

FORM PTO-1390

U.S. Department of Commerce Patent and Trademark Office

Attorney's Docket No.

2328-117

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

U.S. Application No. (if known, see 37 CFR 1.5)

09/529967INTERNATIONAL APPLICATION NO.
PCT/FI98/00873INTERNATIONAL FILING DATE
11 November 1998PRIORITY DATE CLAIMED
14 November 1997**TITLE OF INVENTION**

TETRACYCLINE ASSAY METHOD

APPLICANT(S) FOR DO/EO/US

Matti KORPELA, Matti KARP, and Jussi KURITTU

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11. TO 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☒ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
Small Entity Statement

09/529967

416 Rec'd PCT/PTO 24 APR 2000

2328-117

JLI:ch

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Matti KORPELA et al.)	U.S. National Phase,
)	PCT/FI98/00873
Serial No. (to be assigned))	
)	Intl. Filing Date:
Filed: 24 April 2000)	11 November 1998
)	
For: TETRACYCLINE ASSAY METHOD)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-identified U.S. National Phase of PCT/FI98/00873, filed concurrently herewith, please enter the following amendments thereto:

IN THE CLAIMS:

Please amend the claims as follows:

Claim 3, line 1, delete "or 2".

Claim 5, line 1, delete "or 2".

Claim 7, line 1, change "any of the claims 1-6" to -- claim 1 --.

Claim 8, line 1, change "any of the claims 1-6" to -- claim 1 --.

Claim 9, line 1, change "any of the claims 1-8" to -- claim 1 --.

Claim 10, line 1, change "any of the claims 1-9" to -- claim 1 --.

Claim 13, line 1, delete "or 12,".

Please add the following new claims:

-- 16. The method according to claim 2 characterized in that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO:3), tetracycline repressor (TetR) (SEQ ID NO:11) and tetracycline promoter (TetA) (SEQ ID NO:9) from *Tn10*. --

-- 17. The method according to claim 16 characterized in that the DNA vector is the plasmid pTetLux1 (SEQ ID NO:3). --

-- 18. The method according to claim 2 characterized in that

- the DNA vector is a plasmid containing the insect luciferase gene (SEQ ID NO:1), tetracycline repressor (TetR) (SEQ ID NO:11) and tetracycline promoter (TetA) (SEQ ID NO:9) from *Tn10*, and that

- D-luciferin is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells. --

-- 19. The method according to claim 18 characterized in that the DNA vector is the plasmid pTetLuc1 (SEQ ID NO:1). --

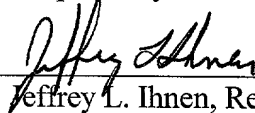
-- 20. The cell according to claim 12 characterized in that it is in dried form, e.g., in lyophilized form. --

REMARKS

The claims have been amended to delete multiple dependencies and to bring them more into conformance with U.S. practice. No new matter has been added by the above amendments, and their entry is therefore requested.

Respectfully submitted,

By



Jeffrey L. Ihnen, Reg. No. 28,957

Attorney for Applicants

ROTHWELL, FIGG, ERNST & KURZ, p.c.

Suite 701-E, 555 13th Street, N.W.

Washington, D.C. 20004

Telephone: (202)783-6040

Dated: 24 April 2000

SMALL ENTITY DECLARATION

APPLICANT OR PATENTEE KORPELA, Matti, KARP, Matti and KURITTU, Jussi

SERIAL NO. _____ ☐ PATENT NO. _____ DOCKET NO. _____

(Check one

1. ☐ FILED OR ISSUED _____

2. ☐ SUBMITTED HEREWITH _____

of blocks

1 or 2)

FOR "Tetracycline assay method"

(Insert Title)

I(we) hereby declare that I(we) am(are) entitled to the benefit of small entity status with respect to the above-identified application or patent for purposes of paying reduced fees under 35 USC 41(a) & (b) to the U.S. Patent and Trademark Office.



A. INDEPENDENT INVENTOR

I(we) qualify as (an) independent inventor(s) as defined in 37 CFR 1.9(c).



B. INDIVIDUAL NON-INVENTOR

I would qualify as an independent inventor as defined in 37 CFR 1.9(c) if I had made the invention.



C. SMALL BUSINESS CONCERN

I am ☐ THE OWNER ☐ AN OFFICIAL of the small business concern identified below and am empowered to act on behalf of the concern. The concern qualifies under 37 CFR 1.9(d) and 13 CFR 121.1301-1305. Rights under contract or law have been conveyed to and remain with the concern and are exclusive unless a checkmark is placed here ☐. All other rights belong to small entities as defined in 37 CFR 1.9.



D. NON-PROFIT ORGANIZATION

I am an official am empowered to act on behalf of the non-profit organization identified below. The organization qualifies under 37 CFR 1.9(e), sub section: ☐ (1) ☐ (2) ☐ (3) ☐ (4). Rights under contract or law have been conveyed to and remain with the organization and are exclusive unless a checkmark is placed here ☐. All other rights belong to small entities as defined in 37 CFR 1.9.

I(we) acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I(we) declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

A. INDEPENDENT INVENTOR(S)

KORPELA, Matti

Name

Signature

Date

KARP, Matti

Name

Signature

Date

KURITTU, Jussi

Name

Signature

Date

Matti Korpe

18.4.2000

Matti Karp

17.4.2000

J. Kurittu

19.4.2000

Tetracycline assay method.

FIELD OF THE INVENTION

This invention relates to a method for the determination of a tetracycline in a sample. The invention also concerns recombinant prokaryotic cells capable of emitting light in response to the existence of a tetracycline in a sample. Furthermore, the invention relates to novel DNA vectors useful for the construction of said prokaryotic cells.

10 BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

- 15 Whole cells can be used in methods based on the use of living cells or organisms as sensor tools of detection. Many of these methods utilize bacterial or yeast cells. Prokaryotic organisms and especially *Escherichia coli* bacterium are very well characterized and maps of genes and their sequences at nucleotide level are known. Therefore the behavior of the whole cell sensor can be better understood. Because
20 of this fact it is also possible to develop analyte or group specific sensors utilizing different regulatory regions of genomes and also various microbial strains.

- Whole cells can be utilized in biosensors which are devices consisting of 1) a sensor, 2) a recording unit and 3) a possible connector such as fiber optic guide
25 between 1 and 2. The recording unit has several choices of what is the physical background of the measurement. It can be change in heat, conductance, color reaction, changes in fluorescent properties, emission of endogenous light from the sensor cells etc.

Antibiotics used as medicines against microbial invasion are detected from body fluids in order to study the dosage and penetration of the medicine. Often the effective therapeutic range of the antibiotic is rather narrow and the risks of overdosage might be too big. It is also important to measure the presence or concentration of antibiotics from meat and milk due to syndrome of allergic people. In the course of cheese production milk used as starting material should not contain antibiotics due to the fact that cheesemaking bacteria are not able to work on contaminated milk.

- 10 Conventional tests for the measurement of toxic substances such as antimicrobial agents (antibiotics) are based on the inhibition of growth. Growth inhibition can be followed by monitoring the zone where the growth of microbes is inhibited on a nutrient agar plate around a disk onto which an antibiotic dilution was pipetted.
- 15 Typical examples of agar diffusion tests are cylindrical, hole or disk methods. The difference in these tests is only restricted in the way the sample is applied on the agar and also the way the bacteria in the test is used. Another means is to follow the metabolism of the test organisms by estimating the intensity of a color reaction which is affected by the inhibitory antibiotic present and comparing it to the
- 20 uninhibited control (e.g. the commercial products: Delvo TestTM, Brilliant black-reduction test, Charm Farm Test, Charm AIM-96 and Valio T101-test). Since microbiological methods utilize bacteria or their spores it is the sensitivity of the test bacteria which is of utmost importance. Thus far one had to make compromises in the choice of a suitable test strain since great sensitivity against antimicrobial agents
- 25 and other characteristics needed for the test strain have not been common features for the same strain of bacteria. A major drawback when using microbes in antibiotic residue tests is slow and unsensitive performance. Since in these methods one always controls in a way or other the growth of the tester strain one cannot imagine

the test to be performed in an hour. This is due to the fact that the growth of the microbe is a slow phenomenon even at its fastest mode. Also in many cases microbes are in spores or freeze-dried, the regeneration of which makes the tests even more slow to perform.

5

OBJECT AND SUMMARY OF THE INVENTION

The object of the invention is to provide a novel method of determining a tetracycline in a sample where said method is rapid and selective for tetracyclines, i.e. the method is able to distinguish tetracyclines from other antimicrobial agents.

10

According to one aspect of the invention a method for the determination of a tetracycline in a sample is provided, wherein the method is characterized in that

15

- the sample is brought into contact with prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,
 - detecting the luminescence emitted from the cells, and
 - comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline
 - wherein a detectable luminescence higher than a luminescence of the control
- 20 indicates the presence of tetracycline in the sample.

25

According to another aspect, the invention concerns a recombinant prokaryotic cell which encompasses a DNA vector including a nucleotide sequence encoding a light producing enzyme, tetracycline repressor and tetracycline promoter.

According to yet another aspect, the invention concerns a plasmid which comprises either

- the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*, or
- the insect luciferase gene (SEQ ID NO: 1), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a shows schematically the method according to this invention, where cells cloned with the plasmid pTetLux1 (SEQ ID NO: 3) are used.

- 10 Figure 1b shows schematically the method according to this invention, where cells cloned with the plasmid pTetLuc1 (SEQ ID NO: 1) are used.

Figure 1c shows schematically the production of the luciferase enzyme,

- 15 Figure 2 shows the plasmid pTetLux1 (SEQ ID NO: 3).

Figure 3 shows the plasmid pTetLuc1 (SEQ ID NO: 1).

- 20 Figure 4a shows the production of light (induction factor) versus concentration of tetracycline in samples for three different tetracyclines,

Figure 4b shows the production of light (induction factor) versus concentration of tetracycline in samples for further four different tetracyclines.

- 25 Figure 5 shows the effect of magnesium ions on the sensitivity of the method according to the invention.

Figure 6 illustrates possibilities of changing the assay window for the method of the invention by adjusting magnesium ion concentration and pH.

Figure 7 shows the induction factor versus tetracycline concentration when using freeze-dried *E. coli* in the determination of tetracycline.

Figure 8 shows a comparison of the assays based on using cells with the plasmid pTetLuc1 (SEQ ID NO: 1) and with the plasmid pTetLux1 (SEQ ID NO: 3).

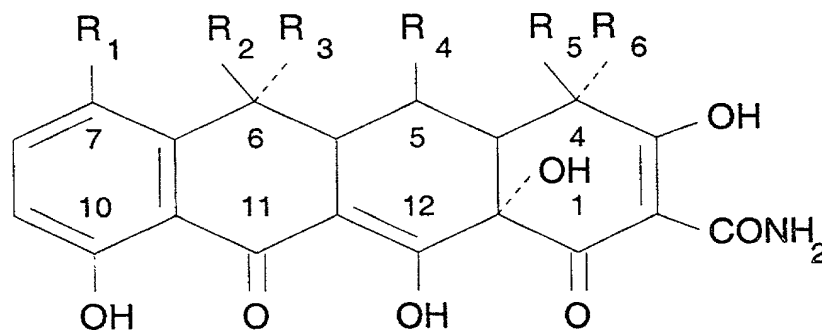
Figure 9 shows induction factors versus antibiotic concentrations of a pig serum sample (cells *E. coli* K12, pTetLux1).

Figure 10 shows the effect of EDTA in a milk sample assay, and

Figure 11 shows the light emission versus time for an assay according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

The term "tetracycline" shall be understood to include any compound covered by the general structure formula



and particularly the specific commercially available compounds listed in the table below.

GENERIC NAME	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
Chlorotetracycline	Cl	OH	CH ₃	H	H	N(CH ₃) ₂
Demethylchlorotetracycline	Cl	OH	H	H	H	N(CH ₃) ₂
Doxycycline	H	H	CH ₃	OH	H	N(CH ₃) ₂
Methacycline	H	CH ₃	H	OH	H	N(CH ₃) ₂
Minocycline	N(CH ₃) ₂	H	H	H	H	N(CH ₃) ₂
Oxytetracycline	H	OH	CH ₃	OH	H	N(CH ₃) ₂
Tetracycline	H	OH	CH ₃	H	H	N(CH ₃) ₂

Furthermore, the term "tetracycline" shall be understood to cover the metabolic and other reformulation/decomposition products thereof.

5

The cells useful in the method of the invention are preferably *Escherichia coli*, which are stored in dried form, e.g. in lyophilized form before their use in the method according to the invention. Also freshly cultivated cells can be used.

- 10 According to a preferred embodiment, the DNA vector including a nucleotide sequence encoding a light producing enzyme is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from transposon *Tn10*. Particularly preferable is the plasmid pTetLux1 (SEQ ID NO: 3).

15

According to another preferred embodiment, the DNA vector including a nucleotide sequence encoding a light producing enzyme is a plasmid containing the insect

luciferase gene, tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*. In this case the substrate for insect luciferase reaction, D-luciferin, is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells. The plasmid is preferably pTetLuc1
5 (SEQ ID NO: 1).

The method according to this invention is useful for the determination of tetracycline in various kinds of samples. As examples can be mentioned milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma, whole blood or the
10 like.

The luminescence of the cells is preferably measured using an X-ray or polaroid film, a CCD-camera (Charge Coupled Device), a liquid scintillation counter or, most preferably, a luminometer.
15

The sensitivity of this analysis method with respect to the tetracycline can be controlled by increasing or decreasing the concentration of divalent metal ions, e.g. magnesium ions, in the mixture of the sample and the cells, by adjusting the pH or by combined adjusting of the divalent metal ion concentration and the pH.
20 Increasing concentration of magnesium ions decreases the sensitivity and vice versa. Increasing pH will also cause a decreasing sensitivity. The sensitivity of the analysis with respect to the tetracycline can be increased by the use of cells which are especially antibiotic sensitive mutant strains. Chelating agents such as EDTA can be added to further sensitize the sensor system for tetracyclines.

25

Figures 1 show a schematic representation of a method based on specific detection of the presence of tetracyclines using microbial cells cloned with either the plasmid pTetLux1 (SEQ ID NO: 3) (Figure 1a) or with the plasmid pTetLuc1 (SEQ ID

NO: 1) (Figure 1b). The figures show that cells containing either of the plasmids can be triggered to produce light by adding a chemical agent (a tetracycline). Light production is a consequence of tetracycline responsive promoter activation due to removal of the tet-repressor protein (SEQ ID NO: 11) leading to the production of
5 luciferase specific mRNA and luciferase protein (SEQ ID NO: 2, 4-8) itself. The principle is demonstrated in Figure 1c. In case of the usage of full length bacterial luciferase operon (SEQ ID NO: 3) containing *luxC*, *luxD*, *luxA*, *luxB* and *luxE* genes (SEQ ID NO: 3) (Figure 1a), one is able to get light emission without addition of any substance. In case of insect (e.g. firefly) luciferase (SEQ ID NO: 2) (Figure
10 1b), light is emitted only after the addition of D-luciferin. It should be noticed that the triggering of luciferase synthesis and light production commences immediately when the cells are introduced to the inducer molecules (tetracyclines). Therefore there is no need to use dividing cells and hence there is no need to use long cultivation of microbial cells such as the case is with conventional methods.
15 Therefore, if needed, one can get results in minutes rather than in hours or days which is the case when conventional methods are used.

Figure 2a shows the plasmid pTetLux1 (SEQ ID NO: 3), in which the production of bacterial luciferase (SEQ ID NO: 4-8) of *Photorhabdus luminescens* (formerly
20 *Xenorhabdus luminescens*; the lux-operon structure and the full-length nucleotide sequence of *P. luminescens* was published in Szittner, R. and Meighen, E. (1990) J. Biol. Chem. 265, 16581-16587) can be switched on by the addition of a chemical agent belonging to the tetracycline family of antimicrobial agents in a cloned *E. coli* bacterium. SEQ ID NO: 3 shows the nucleotide sequence of the plasmid pTetLux1.
25 This plasmid construct is devised to contain the five genes from *P. luminescens* luciferase operon necessary for the light production without any additions of substrates, i.e. cells cloned with such a construct produce substrates endogenously. By incubating *E. coli* cells containing this plasmid (or any other microbial strain

wherein a similar regulation/reporter gene system is incorporated containing the necessary secondary regulatory sequences in the constructs such as correct ribosome binding region, transcriptional termination etc.) in the presence of very small amounts of tetracyclines one is able to obtain light production the intensity of which is proportional to the concentration of tetracycline used.

Any *E. coli* mutant strain and especially those strains having a mutation in the export/import machinery of the membranes or otherwise leaky character making it possible for large molecules to easily penetrate inside the cell would be beneficial to use in the method described in this invention. Also other gram-negative bacteria such as strains belonging to genus *Salmonella*, *Shigella*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Erwinia*, *Pseudomonas*, *Serratia* as well as gram-positive organisms such as those belonging to genus *Bacillus* (especially *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. globigii*, *B. natto*, *B. amyloliquefaciens* as well as *B. niger*, *B. brevis*, *B. megaterium*), *Streptomyces*, *Lactobacillus* (especially *L. lactis*, *L. casei*) and *Streptococcus* (especially *S. thermophilus*, *S. cremoris*, *S. agalactiae*) come into question. Especially asporogenic strains of *Bacilli* or *Lactobacilli* are suitable.

Figure 3 shows the plasmid pTetLuc1 (SEQ ID NO: 1), in which the production of firefly luciferase (SEQ ID NO: 2) of *Photinus pyralis* (The gene encoding firefly luciferase was originally cloned and sequenced in the middle of the 1980's by DeWet, J. et al. (1987) Mol. Cell. Biol. 7, 725-737) can be switched on by the addition of a chemical agent belonging to the tetracycline family of antimicrobial agents in a cloned *E. coli* bacterium. SEQ ID NO: 1 shows the nucleotide sequence of this plasmid. By incubating *E. coli* cells containing this plasmid (or any other microbial strain wherein a similar regulation/reporter gene system is incorporated containing the necessary secondary regulatory sequences in the constructs such as correct ribosome binding region, transcriptional termination etc.) in the presence of

very small amounts of tetracyclines one is able to obtain light production by the addition of D-luciferin, which is the substrate of firefly luciferase. The intensity of light emission is proportional to the concentration of tetracycline used.

- 5 Figures 4a and 4b shows the effect of altogether seven different tetracyclines on the production of light as a function of concentration of each tetracycline. As controls different non-tetracycline antibiotics were included in this study to show that the sensor strain is specific for the tetracyclines. The luminescence was emitted from *E. coli* containing the plasmid pTetLux1 (SEQ ID NO: 3). The detection was made
- 10 after an incubation of 90 min. All tetracyclines tested behaved in a very similar manner and induction efficiencies were at the same antibiotic concentration area. This makes this sensor even more attractive for analytical use for the determination of the tetracycline group of antibiotics.
- 15 It should be noted that the accumulation of various tetracyclines into microbial cells is very strongly affected by the extracellular concentration of Mg^{2+} ions. Figure 5 shows the effect of increasing concentrations of Mg^{2+} ions on the behavior of *E. coli* cells containing the plasmid pTetLux1 (SEQ ID NO: 3). As can be seen the tetracycline response curve is shifted to the right as a function of increasing
- 20 concentrations of added Mg^{2+} ions. Thus by increasing the Mg^{2+} ion concentration one is able to decrease the sensitivity of the tetracycline sensor described in this invention. This fact is of great importance in cases where one does not need a high sensitivity of the measurement and where the approximate concentration of the ion is roughly constant and known such as in milk, serum and plasma.
- 25 The sensitivity can be increased by removing magnesium ions from the assay mixture e.g. by adding a chelating agent forming a complex with magnesium.

Figure 6 shows the possibilities to change the assay window for tetracyclines by adjusting the magnesium ion concentration and by combined adjustment of the magnesium ion concentration and pH.

- 5 The sensitivity of the assay can be increased by the use of cells which are especially antibiotic sensitive mutant strains. Hundreds of specific mutations for bacteria are known with which it is possible to study the activity of specific reactions. For instance trace amounts of antibiotics cause visible changes in the metabolism or in the cell membranes of antibiotic sensitive bacterial mutants. Mutations in cell wall
- 10 structural components or biosynthetic enzymes as well as in transport and efflux proteins such as porins might have an effect on the behavior of each sensor. Using these kinds of mutations one is able to develop tests measuring residual antibiotics from biological material very sensitively. It is also rather simple to transfer new characteristics into bacterial cells by genetic engineering techniques. This
- 15 phenomenon broadens the applicability of these organisms in tests utilizing whole cell sensor.

Measurement of light emission can be done by using X-ray or polaroid film, using a liquid scintillation counter, a CCD-camera or a luminometer. The CCD-camera is an

20 instrument which is capable of detecting very low levels of light. In the applications of this invention such kind of a device could be used for the detection of tetracycline residues in food material such as vegetables or meat. The detection of light emission could be directly monitored from the surface of the food material sprayed with engineered luminescent bacteria. Either chemiluminescent (such as peroxidase -

25 luminol) or bioluminescent (such as luciferase - luciferin) reactions can be utilized. The luminometric method is performed with the aid of genes encoding either bacterial or beetle luciferases such as those described in the Figures 2 and 4. Several luminescent bacterial species such as *V. harveyi*, *V. fischeri*, *P. leiognathi*,

- P. phosphoreum*, *Xenorhabdus luminescens* etc. exist. Luminescent beetles are for example *Luciola mingrelica*, *Photinus pyralis*, *Pyrophorus plagiophthalmus*, *Lampyrus noctiluca*, *Pholas dactylus*, etc. Also several eukaryotic species in the sea which luminesce, such as marine ostracod *Vargula hilgendorfii*, jellyfish *Aequorea victoria*, batrachoidid fish *Porichtys notatus*, pempherid fish *Parapriacanthus ransonneti* etc. exist. Fluorescent reporter proteins such as green fluorescent protein (GFP) or any of its variants could be used in the methods described in this invention (Li, X. et al. (1997) J. Biol. Chem. 272, 28545-28549).
- 10 In this invention high detection sensitivity of the luminescent enzyme labels inside a living cell associated with tetracycline-specific induction of label synthesis is based on the use of optimal concentration of all the reactants inside the cell including the necessary cofactors and accessory enzymes. All luciferase genes from these organisms would presumably work in a similar manner as the two examples shown
- 15 in this invention. These systems together with enhancers and modulators (wavelength, emission kinetics etc.) of light emission has been described in more detail in Campbell, A. "Chemiluminescence; principles and applications in biology and medicine", Weinheim; Deerfield Beach, Fl.; VCH; Chichester: Horwood, 1988.
- 20 Peroxidases or oxidases can be used together with compounds such as luminol or acridines (for instance lucigenin) to yield luminescent signals suitable for a detection system described here. Enzymatically generated chemiluminescence offers great sensitivity and rapid detection, too, in assays described in this invention. Thermally stable dioxetanes (such as AMPPD and Lumigen PPD) can be
- 25 enzymatically (such as alkaline phosphatase or β -galactosidase) triggered to produce chemiluminescence (Schaap, A.P. et al. (1989) Clin. Chem. 35, 1863-1864). The only difference to the luciferase enzymes would be that these enzymes are capable

of cleaving a man-made substrate which gives light emission (chemiluminescence) and the luciferases cleave natural substrates to produce light (bioluminescence).

- Tetracycline-controlled expression systems are developed to express heterologous proteins in procaryotic and eucaryotic cells for the purpose of production under a tight control of tet-regulatory system (Skerra, A. (1994) Gene 151, 131-135; Gossen, M. and Bujard, H. (1995) US Patent 5,464,758 ; Lutz, R. and Bujard, H. (1997) Nucleic Acids Res. 25, 1203-1210).
- 10 A method to study various tetracyclines and their mode of action was developed by Chopra et al. (Chopra, I. et al. (1990) Antimicrob. Agents Chemother. 34, 111-116) The assay system developed in this study was based on expression of β -galactosidase gene inserted under the control of tetA-gene. The method resulted in less sensitive detection of tetracyclines compared to the invention described here.
- 15 However in order to obtain maximum sensitivities Chopra et al. showed that it was necessary to add cyclic AMP (cAMP) to the medium which is an extremely expensive molecule to be used in routine applications. Furthermore, the method described by Chopra et al. contains a cell disruption stage by sonication in order to assay for the reporter gene activity, β -galactosidase, which step is not practical.
- 20 Instead, the method described in this invention does not contain any cell disruption. The activity of luciferase can be measured directly from living cells in real-time and in the case of pTetLux1 (SEQ ID NO: 3) there is no need of addition of any substrates. Therefore, promoter activation due to the presense/absense of tetracycline can be monitored continuously.

25

EXPERIMENTS

As cloning hosts and in antibiotic residue measurements various *E. coli* MC1061 (cI+, araD139, Δ (ara-leu)7696, lacX74, galU, galK, hsr, hsm, strA) (Casadaban,

M.J. and Cohen, S.N. (1980) J. Mol. Biol. 138, 179-207), BW322 (CGSC, *rfa210::Tn10*, *thi-1*, *relA1*, *spoT1*, *pyrE*) and K-12 (M72 Sm^R *lacZm-Δbiouvrb*, *trpEA2*, *Nam7Nam53cI857 HI*) (Remaut, E. et al. (1981) Gene 15, 81-93) can be used. Especially the strain LH530 (Hirvas, L. et al. (1997) Microbiology 143, 73-81) which has a decreased rate of lipid A biosynthesis. It has proven to be hypersusceptible to many different antibiotics.

Cells were grown on appropriate minimal agar-plates and were kept maximally one month at +4 °C after which new plates were streaked. The strains were kept also in 15% glycerol at -70 °C, where from growth was started through minimal plates. The cells were first cultivated in 5 ml of 2xTY medium (16 g Bacto tryptone, 8 g Yeast extract, 8 g NaCl, H₂O ad 1 l, pH 7.4, with appropriate antibiotic) 10 h at 30 °C in a shaker after which the cultivation was transferred to a bigger volume for 10 h with same medium.

15

Construction of tetracycline-responsive sensor plasmids:

To construct a recombinant DNA vector carrying luciferase genes under the control of a tetracycline responsive elements two new vectors were created. In the first one modified firefly luciferase gene (SEQ ID NO: 1) from vector pBLuc* (Bonin, A.L. et al. (1994) Gene 141, 75-77) was excised by using restriction enzymes *XbaI* and *HinDIII* and the 1.7 kb fragment was isolated from LGT-agarose gel and purified using Qiagen gel extraction kit. This DNA-fragment containing the entire *Photinus pyralis* luciferase gene (SEQ ID NO: 1) was ligated using T4-DNA-ligase enzyme to vector pASK75 (Skerra, A. (1994) Gene 151, 131-135) which was previously restricted with the same restriction enzymes *XbaI* and *HinDIII* and calf intestinal phosphatase treated to remove the protruding phosphate groups in order to prevent self-ligation. The resulting ligation mixture was incubated 3 hours at room temperature after which one µl of the mixture was electroporated according to

Dower *et al.* (Dower, W.J. et al. (1988) Nucleic Acids Res. 16, 6126-6144) into electrocompetent *E. coli* MC1061 cells. A plasmid was extracted from one of the colonies obtained and checked for the estimated structure by appropriate restriction enzyme digestions and agarose gel electrophoretic techniques. The plasmid obtained
5 was named as pTetLuc1 (SEQ ID NO: 1).

The plasmid containing the luxCDABE genes (SEQ ID NO: 3) of *Photorhabdus luminescens* under the control of tetracycline responsive element was created as follows: Plasmid pASK75 was cut with restriction enzyme *EcoRI* and CIP-treated.
10 The linearized plasmid was separated on a LGT-agarose gel electrophoresis and the agarose was removed by using the Qiagen kit. The lux operon was excised with *EcoRI* from plasmid pCGLS-11 (Frackman, S. et al. (1990) J. Bacteriol. 172, 5767-5773), gel purified as above and ligated to pASK75 by using T4-DNA-ligase at 16 °C overnight. The ligation mixture was electroporated into *E. coli* MC1061 cells as
15 described above and correct transformants were screened for their ability to produce light (as measured with a BioOrbit 1250 manual luminometer) which production was increased in the presence of 1 µg/ml of tetracycline-HCl. The plasmid was further verified by restriction enzyme digestions and the correct structure was named as pTetLux1 (SEQ ID NO: 3). All the DNA-manipulations were performed
20 according to Sambrook *et al.*, "Molecular Cloning: A laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

The vector pASK75 was utilized in the construction of tet-sensor plasmids shown in this invention. The vector pASK75 was originally developed for protein production
25 and purification purposes. It contains a signal sequence for secretion of the recombinant protein into the periplasmic space of *E. coli*. Also a C-terminal fusion between a purification tail, the Strept-tag, was incorporated into the vector to facilitate purification of recombinant protein using streptavidin affinity agarose gel

chromatography. The element controlling recombinant gene expression in the vector is tetA promoter/operator system that allows efficient regulation of the expression, which in Skerra's paper was described for the production and one-step purification of a murine single-chain antibody fragment. The tetA promoter/operator (SEQ ID NO: 9) is controlled by tetR-repressor (SEQ ID NO: 9) which is produced by the corresponding gene (SEQ ID NO: 9). Some of the above mentioned elements were eliminated from the present plasmids due to unnecessary features with respect to this invention.

10 Transfer of the tetracycline sensor vectors to the antibiotic sensitive *E. coli* strain:

Either pTetLux1 (SEQ ID NO: 3) or pTetLuc1 (SEQ ID NO: 1) was transformed into *E. coli* LH530 cells by electroporation as described above. The transformed cells were restreaked on agar plates and kept maximally for 2 weeks at +4 °C after which a new plate was streaked.

Use of the manipulated *E. coli* in tetracycline determination methods:

Example 1

Freeze-dried *E. coli* K-12/pTetLux1 were reconstituted with 1.0 ml of L-broth and bacteria were diluted 1:10 with 25 mM MES buffer in M9 minimal medium, pH 6.0. 190 µl bacterial suspension was added to microtiter plate wells containing 10 µl of tetracycline dilutions. The plate was incubated 90 minutes at 37 °C after which the plate was measured with Labsystems Luminoskan luminometer. As seen from Figure 7 the sensitivity of the assay of tetracycline is very high and comparable to that of fresh cells.

Example 2

Two different types of sensor DNA vector construct were compared. Strains *E. coli* K-12/pTetLux1 and *E. coli* K-12/pTetLuc1 were cultivated in L-broth media until optical density measured at 600 nm (OD600) was 1.5. The cells were diluted 1 to 50
5 with 25 mM MES-buffer in M9 minimal medium, pH 6.0 (Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory, Cold Spring Harbor) and 190 µl was added to microtitration plate wells and 10 µl of sample dilution of tetracycline was added. After a 60 min incubation at 37 °C the light emission was measured using a Labsystems Luminoskan luminometer. Figure 8 shows the bioluminescence dose
10 response curve as a function of tetracycline added. As seen from the figure both systems (bacterial and insect luciferase) give roughly equal sensitivity of tetracycline detection.

One is able to use different luciferases instead of bacterial luciferase (SEQ ID
15 NO: 4-8) from *P. luminescens* without losing sensitivity or other performance of the test. Figure 8 shows an analogous measurement to the one in Figure 4b. In the plasmid used in this test (pTetLuc1) the bacterial luciferase was compensated with firefly luciferase (SEQ ID NO: 2) as described in Figure 3. The test was done essentially as with bacterial luciferase except that after the cells had been incubated
20 with or without tetracycline 10 minutes at 37 °C the cells were measured for light production after 15 minutes incubation time at 37 °C by adding 100 µl of solution containing 1 mM D-luciferin, in 0.1 M Na-citrate buffer, pH 5.0. Thereafter light production was measured using a manual luminometer 1250 (LKB-Wallac, Turku, Finland). As can be seen from Figure 8 sensitivity of the method to detect
25 tetracycline hydrochloride is extremely high and comparable to the detection made with bacterial luciferase.

Example 3

A lipemic pig serum was spiked at different concentrations of tetracycline, chlorotetracycline and oxytetracycline. Fresh *E. coli* K-12/pTetLux1 were diluted 1:50 with 25 mM MES buffer in M9 minimal medium, pH 6.0. 100 µl bacterial suspension was added to microtiter plate wells containing 100 µl of pig serum spiked with different tetracyclines. The plate was incubated 90 minutes at 37 °C after which the plate was measured with Labsystems Luminoskan luminometer. As seen from Figure 9 the sensitivity of the assay of different tetracyclines in pig serum matrix is very high.

10

Example 4

Tetracyclines will form chelate complexes with Ca^{2+} and Mg^{2+} in samples (e.g. milk), and lose their antimicrobial and induction activity in our assay system. Tetracyclines can be displaced from cation chelates by using strong chelating agents such as EDTA. Figure 10 shows the determination of tetracycline from a milk sample, which is spiked with different concentrations of tetracycline. Different amounts of EDTA were added to milk samples and this kind of displacement of cation-tetracycline complex clearly improved the sensitivity of the assay. In the assay we used freeze-dried *E. coli* K12/pTetLux1 that were reconstituted with L-broth 10 minutes in room temperature before the assay.

20

Example 5

Figure 11 shows the kinetics of bacterial bioluminescence after exposure of *E. coli* K-12/pTetLux1 to different dilutions of tetracycline antibiotics. The specific induction of tetracycline is very fast and specific light emission is seen already at the 10 minutes measuring point in the assay.

25

[illegible]

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: KORPELA, Matti
(B) STREET: Maijamaentie 13
(C) CITY: Naantali
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): FIN-21100

(A) NAME: KARP, Matti
(B) STREET: Kampakatu 1
(C) CITY: Kaarina
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): FIN-20660

(A) NAME: KURITTU, Jussi
(B) STREET: Puutarhakatu 16 A 20
(C) CITY: Turku
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): FIN-20100

(ii) TITLE OF INVENTION: A NEW ASSAY METHOD

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: FI 974235
(B) FILING DATE: 14-NOV-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4846 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Photinus pyralis

(vii) IMMEDIATE SOURCE:

(B) CLONE: pTetLuc1

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: Plasmid

004470 9652560

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:1..3098
- (D) OTHER INFORMATION:/standard_name= "Vector pASK75"
/note= "Part of plasmid originating from vector pASK75;
feature description below, SEQ ID 9-11."
/citation= ([2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:3119..4768
- (D) OTHER INFORMATION:/product= "Photinus pyralis
luciferase"
/citation= ([1])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Bonin,
- (B) TITLE: Photinus pyralis luciferase: vectors that
contain a modified luc coding sequence allowing
convenient transfer into other systems
- (C) JOURNAL: Gene
- (D) VOLUME: 141
- (F) PAGES: 75-77
- (G) DATE: 1994
- (K) RELEVANT RESIDUES IN SEQ ID NO: 1: FROM 3099 TO 4772

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Skerra, A
- (B) TITLE: Use of the tetracycline promoter for the
tightly regulated production of a murine antibody
fragment in Escherichia coli
- (C) JOURNAL: Gene
- (D) VOLUME: 151
- (E) ISSUE: 1-2
- (F) PAGES: 131-135
- (G) DATE: 30-DEC-1994
- (K) RELEVANT RESIDUES IN SEQ ID NO: 1: FROM 1 TO 3098

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```

AGCTTGACCT GTGAAGTGAA AAATGGCGCA CATTGTGCGA CATTTTTTTT GTCTGCCGTT      60
TACCGCTACT GCGTCACGGA TCTCCACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT      120
GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC      180
GCTTTCTTCC CTTCCTTTCT CGCCACGTTT GCCGGCTTTC CCCGTCAAGC TCTAAATCGG      240
GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT      300
TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG      360
TTGGAGTCCA CGTTCTTTAA TAGTGGA CTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT      420
ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA      480
AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT      540
TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA      600
CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA      660
AAAAGGAAGA GTATGAGTAT TCAACATTTT CGTGTGCGCC TTATTCCTTT TTTTGCGGCA      720
TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT      780
CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG      840

```

AGTTTTCGCC	CCGAAGAACG	TTTTCCAATG	ATGAGCACTT	TTAAAGTTCT	GCTATGTGGC	900
GCGGTATTAT	CCCGTATTGA	CGCCGGGCAA	GAGCAACTCG	GTCGCCGCAT	ACACTATTCT	960
CAGAATGACT	TGGTTGAGTA	CTCACCAGTC	ACAGAAAAGC	ATCTTACGGA	TGGCATGACA	1020
GTAAGAGAAT	TATGCAGTGC	TGCCATAACC	ATGAGTGATA	ACACTGCGGC	CAACTTACTT	1080
CTGACAACGA	TGGGAGGACC	GAAGGAGCTA	ACCGCTTTTT	TGCACAACAT	GGGGGATCAT	1140
GTAATCGCC	TTGATCGTTG	GGAACCGGAG	CTGAATGAAG	CCATACCAA	CGACGAGCGT	1200
GACACCACGA	TGCCTGTAGC	AATGGCAACA	ACGTTGCGCA	AACTATTAA	TGGCGAACTA	1260
CTTACTCTAG	CTTCCCGGCA	ACAATTGATA	GA CTGGATGG	AGGCGGATAA	AGTTGCAGGA	1320
CCACTTCTGC	GCTCGGCCCT	TCCGGCTGGC	TGGTTTATTG	CTGATAAATC	TGGAGCCGGT	1380
GAGCGTGGCT	CTCGCGGTAT	CATTGCAGCA	CTGGGGCCAG	ATGGTAAGCC	CTCCCGTATC	1440
GTAGTTATCT	ACACGACGGG	GAGTCAGGCA	ACTATGGATG	AACGAAATAG	ACAGATCGCT	1500
GAGATAGGTG	CCTCACTGAT	TAAGCATTTG	TAGGAATTAA	TGATGTCTCG	TTTAGATAAA	1560
AGTAAAGTGA	TTAACAGCGC	ATTAGAGCTG	CTTAATGAGG	TCGGAATCGA	AGGTTTAACA	1620
ACCCGTAAAC	TCGCCCAGAA	GCTAGGTGTA	GAGCAGCCTA	CATTGTATTG	GCATGTAAAA	1680
AATAAGCGGG	CTTTGCTCGA	CGCCTTAGCC	ATTGAGATGT	TAGATAGGCA	CCATACTCAC	1740
TTTTTGCCCTT	TAGAAGGGGA	AAGCTGGCAA	GATTTTTTTAC	GTAATAACGC	TAAAAGTTTT	1800
AGATGTGCTT	TACTAAGTCA	TCGCGATGGA	GCAAAAGTAC	ATTTAGGTAC	ACGGCCTACA	1860
GAAAAACAGT	ATGAAACTCT	CGAAAATCAA	TTAGCCTTTT	TATGCCAACA	AGGTTTTTCA	1920
CTAGAGAATG	CATTATATGC	ACTCAGCGCA	GTGGGGCATT	TTACTTTAGG	TTGCGTATTG	1980
GAAGATCAAG	AGCATCAAGT	CGCTAAAGAA	GAAAGGGAAA	CACCTACTAC	TGATAGTATG	2040
CCGCCATTAT	TACGACAAGC	TATCGAATTA	TTTGATCACC	AAGGTGCAGA	GCCAGCCTTC	2100
TTATTCGGCC	TTGAATTGAT	CATATGCGGA	TTAGAAAAAC	AACTTAAATG	TGAAAGTGGG	2160
TCTTAAAGC	AGCATAACCT	TTTTCCGTGA	TGGTAACTTC	ACTAGTTTAA	AAGGATCTAG	2220
GTGAAGATCC	TTTTTGATAA	TCTCATGACC	AAAATCCCTT	AACGTGAGTT	TTCGTTCCAC	2280
TGAGCGTCAG	ACCCCGTAGA	AAAGATCAAA	GGATCTTCTT	GAGATCCTTT	TTTTCTGCGC	2340
GTAATCTGCT	GCTTGCAAAC	AAAAAAACCA	CCGCTACCAG	CGGTGGTTTG	TTTGCCGGAT	2400
CAAGAGCTAC	CAACTCTTTT	TCCGAAGGTA	ACTGGCTTCA	GCAGAGCGCA	GATACCAAAT	2460
ACTGTCCTTC	TAGTGTAGCC	GTAGTTAGGC	CACCACTTCA	AGAACTCTGT	AGCACCGCCT	2520
ACATACCTCG	CTCTGCTAAT	CCTGTTACCA	GTGGCTGCTG	CCAGTGGCGA	TAAGTCGTGT	2580
CTTACCGGGT	TGGACTCAAG	ACGATAGTTA	CCGATAAGG	CGCAGCGGTC	GGGCTGAACG	2640
GGGGGTTTCG	GCACACAGCC	CAGCTTGAG	CGAACGACCT	ACACCGAACT	GAGATACCTA	2700
CAGCGTGAGC	TATGAGAAAG	CGCCACGCTT	CCCGAAGGGA	GAAAGGCGGA	CAGGTATCCG	2760
GTAAGCGGCA	GGGTCGGAAC	AGGAGAGCGC	ACGAGGGAGC	TTCCAGGGGG	AAACGCCTGG	2820
TATCTTTATA	GTCCTGTCGG	GTTTCGCCAC	CTCTGACTTG	AGCGTCGATT	TTTGTGATGC	2880

TCGTCAGGGG GCGCGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTTT ACGGTTCCCTG	2940
GCCTTTTGCT GGCCTTTTGC TCACATGACC CGACACCATC GAATGGCCAG ATGATTAATT	3000
CCTAATTTTTT GTTGACACTC TATCATTGAT AGAGTTATTT TACCACTCCC TATCAGTGAT	3060
AGAGAAAAGT GAAATGAATA GTTCGACAAA AATCTAGAAC TAGTGGATCC CCCGTACC	3118
ATG GAA GAC GCC AAA AAC ATA AAG AAA GGC CCG GCG CCA TTC TAT CCG Met Glu Asp Ala Lys Asn Ile Lys Lys Gly Pro Ala Pro Phe Tyr Pro 1 5 10 15	3166
CTA GAG GAT GGA ACC GCT GGA GAG CAA CTG CAT AAG GCT ATG AAG AGA Leu Glu Asp Gly Thr Ala Gly Glu Gln Leu His Lys Ala Met Lys Arg 20 25 30	3214
TAC GCC CTG GTT CCT GGA ACA ATT GCT TTT ACA GAT GCA CAT ATC GAG Tyr Ala Leu Val Pro Gly Thr Ile Ala Phe Thr Asp Ala His Ile Glu 35 40 45	3262
GTG AAC ATC ACG TAC GCG GAA TAC TTC GAA ATG TCC GTT CGG TTG GCA Val Asn Ile Thr Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala 50 55 60	3310
GAA GCT ATG AAA CGA TAT GGG CTG AAT ACA AAT CAC AGA ATC GTC GTA Glu Ala Met Lys Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val 65 70 75 80	3358
TGC AGT GAA AAC TCT CTT CAA TTC TTT ATG CCG GTG TTG GGC GCG TTA Cys Ser Glu Asn Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu 85 90 95	3406
TTT ATC GGA GTT GCA GTT GCG CCC GCG AAC GAC ATT TAT AAT GAA CGT Phe Ile Gly Val Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg 100 105 110	3454
GAA TTG CTC AAC AGT ATG AAC ATT TCG CAG CCT ACC GTA GTG TTT GTT Glu Leu Leu Asn Ser Met Asn Ile Ser Gln Pro Thr Val Val Phe Val 115 120 125	3502
TCC AAA AAG GGG TTG CAA AAA ATT TTG AAC GTG CAA AAA AAA TTA CCA Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro 130 135 140	3550
ATA ATC CAG AAA ATT ATT ATC ATG GAT TCT AAA ACG GAT TAC CAG GGA Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly 145 150 155 160	3598
TTT CAG TCG ATG TAC ACG TTC GTC ACA TCT CAT CTA CCT CCC GGT TTT Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe 165 170 175	3646
AAT GAA TAC GAT TTT GTA CCA GAG TCC TTT GAT CGT GAC AAA ACA ATT Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile 180 185 190	3694
GCA CTG ATA ATG AAC TCC TCT GGA TCT ACT GGG TTA CCT AAG GGT GTG Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val 195 200 205	3742
GCC CTT CCG CAT AGA ACT GCC TGC GTC AGA TTC TCG CAT GCC AGA GAT Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp 210 215 220	3790
CCT ATT TTT GGC AAT CAA ATC ATT CCG GAT ACT GCG ATT TTA AGT GTT Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val 225 230 235 240	3838

GTT CCA TTC CAT CAC GGT TTT GGA ATG TTT ACT ACA CTC GGA TAT TTG Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu 245 250 255	3886
ATA TGT GGA TTT CGA GTC GTC TTA ATG TAT AGA TTT GAA GAA GAG CTG Ile Cys Gly Phe Arg Val Val Leu Met Tyr Arg Phe Glu Glu Leu 260 265 270	3934
TTT TTA CGA TCC CTT CAG GAT TAC AAA ATT CAA AGT GCG TTG CTA GTA Phe Leu Arg Ser Leu Gln Asp Tyr Lys Ile Gln Ser Ala Leu Leu Val 275 280 285	3982
CCA ACC CTA TTT TCA TTC TTC GCC AAA AGC ACT CTG ATT GAC AAA TAC Pro Thr Leu Phe Ser Phe Phe Ala Lys Ser Thr Leu Ile Asp Lys Tyr 290 295 300	4030
GAT TTA TCT AAT TTA CAC GAA ATT GCT TCT GGG GGC GCA CCT CTT TCG Asp Leu Ser Asn Leu His Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser 305 310 315 320	4078
AAA GAA GTC GGG GAA GCG GTT GCA AAA CGC TTC CAT CTT CCA GGG ATA Lys Glu Val Gly Glu Ala Val Ala Lys Arg Phe His Leu Pro Gly Ile 325 330 335	4126
CGA CAA GGA TAT GGG CTC ACT GAG ACT ACA TCA GCT ATT CTG ATT ACA Arg Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Ile Leu Ile Thr 340 345 350	4174
CCC GAG GGG GAT GAT AAA CCG GGC GCG GTC GGT AAA GTT GTT CCA TTT Pro Glu Gly Asp Asp Lys Pro Gly Ala Val Gly Lys Val Val Pro Phe 355 360 365	4222
TTT GAA GCG AAG GTT GTG GAT CTG GAT ACC GGG AAA ACG CTG GGC GTT Phe Glu Ala Lys Val Val Asp Leu Asp Thr Gly Lys Thr Leu Gly Val 370 375 380	4270
AAT CAG AGA GGC GAA TTA TGT GTC AGA GGA CCT ATG ATT ATG TCC GGT Asn Gln Arg Gly Glu Leu Cys Val Arg Gly Pro Met Ile Met Ser Gly 385 390 395 400	4318
TAT GTA AAC AAT CCG GAA GCG ACC AAC GCC TTG ATT GAC AAG GAT GGA Tyr Val Asn Asn Pro Glu Ala Thr Asn Ala Leu Ile Asp Lys Asp Gly 405 410 415	4366
TGG CTA CAT TCT GGA GAC ATA GCT TAC TGG GAC GAA GAC GAA CAC TTC Trp Leu His Ser Gly Asp Ile Ala Tyr Trp Asp Glu Asp Glu His Phe 420 425 430	4414
TTC ATA GTT GAC CGC TTG AAG TCT TTA ATT AAA TAC AAA GGA TAC CAG Phe Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln 435 440 445	4462
GTG GCC CCC GCT GAA TTG GAG TCG ATA TTG TTA CAA CAC CCC AAC ATC Val Ala Pro Ala Glu Leu Glu Ser Ile Leu Leu Gln His Pro Asn Ile 450 455 460	4510
TTC GAC GCG GGC GTG GCA GGT CTT CCC GAC GAT GAC GCC GGT GAA CTT Phe Asp Ala Gly Val Ala Gly Leu Pro Asp Asp Asp Ala Gly Glu Leu 465 470 475 480	4558
CCC GCC GCC GTT GTT GTT TTG GAG CAC GGA AAG ACG ATG ACG GAA AAA Pro Ala Ala Val Val Val Leu Glu His Gly Lys Thr Met Thr Glu Lys 485 490 495	4606
GAG ATC GTG GAT TAC GTC GCC AGT CAA GTA ACA ACC GCC AAA AAG TTG Glu Ile Val Asp Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu 500 505 510	4654

CGC GGA GGA GTT GTG TTT GTG GAC GAA GTA CCG AAA GGT CTT ACC GGA 4702
 Arg Gly Gly Val Val Phe Val Asp Glu Val Pro Lys Gly Leu Thr Gly
 515 520 525

AAA CTC GAC GCA AGA AAA ATC AGA GAG ATC CTC ATA AAG GCC AAG AAG 4750
 Lys Leu Asp Ala Arg Lys Ile Arg Glu Ile Leu Ile Lys Ala Lys Lys
 530 535 540

GGC GGA AAG TCC AAA TTG TAAAATGTAA CTGTATTTCAG CGATGACGAA 4798
 Gly Gly Lys Ser Lys Leu
 545 550

ATTCTTAGCT ATTGTAATAC TCTAGCGGGC TGCAGGAATT CGATATCA 4846

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 550 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Glu Asp Ala Lys Asn Ile Lys Lys Gly Pro Ala Pro Phe Tyr Pro
 1 5 10 15

Leu Glu Asp Gly Thr Ala Gly Glu Gln Leu His Lys Ala Met Lys Arg
 20 25 30

Tyr Ala Leu Val Pro Gly Thr Ile Ala Phe Thr Asp Ala His Ile Glu
 35 40 45

Val Asn Ile Thr Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala
 50 55 60

Glu Ala Met Lys Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val
 65 70 75 80

Cys Ser Glu Asn Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu
 85 90 95

Phe Ile Gly Val Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg
 100 105 110

Glu Leu Leu Asn Ser Met Asn Ile Ser Gln Pro Thr Val Val Phe Val
 115 120 125

Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro
 130 135 140

Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly
 145 150 155 160

Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe
 165 170 175

Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile
 180 185 190

Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val
 195 200 205

Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp
 210 215 220

Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val
 225 230 235 240
 Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu
 245 250 255
 Ile Cys Gly Phe Arg Val Val Leu Met Tyr Arg Phe Glu Glu Glu Leu
 260 265 270
 Phe Leu Arg Ser Leu Gln Asp Tyr Lys Ile Gln Ser Ala Leu Leu Val
 275 280 285
 Pro Thr Leu Phe Ser Phe Phe Ala Lys Ser Thr Leu Ile Asp Lys Tyr
 290 295 300
 Asp Leu Ser Asn Leu His Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser
 305 310 315 320
 Lys Glu Val Gly Glu Ala Val Ala Lys Arg Phe His Leu Pro Gly Ile
 325 330 335
 Arg Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Ile Leu Ile Thr
 340 345 350
 Pro Glu Gly Asp Asp Lys Pro Gly Ala Val Gly Lys Val Val Pro Phe
 355 360 365
 Phe Glu Ala Lys Val Val Asp Leu Asp Thr Gly Lys Thr Leu Gly Val
 370 375 380
 Asn Gln Arg Gly Glu Leu Cys Val Arg Gly Pro Met Ile Met Ser Gly
 385 390 395 400
 Tyr Val Asn Asn Pro Glu Ala Thr Asn Ala Leu Ile Asp Lys Asp Gly
 405 410 415
 Trp Leu His Ser Gly Asp Ile Ala Tyr Trp Asp Glu Asp Glu His Phe
 420 425 430
 Phe Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln
 435 440 445
 Val Ala Pro Ala Glu Leu Glu Ser Ile Leu Leu Gln His Pro Asn Ile
 450 455 460
 Phe Asp Ala Gly Val Ala Gly Leu Pro Asp Asp Asp Ala Gly Glu Leu
 465 470 475 480
 Pro Ala Ala Val Val Val Leu Glu His Gly Lys Thr Met Thr Glu Lys
 485 490 495
 Glu Ile Val Asp Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu
 500 505 510
 Arg Gly Gly Val Val Phe Val Asp Glu Val Pro Lys Gly Leu Thr Gly
 515 520 525
 Lys Leu Asp Ala Arg Lys Ile Arg Glu Ile Leu Ile Lys Ala Lys Lys
 530 535 540
 Gly Gly Lys Ser Lys Leu
 545 550

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10220 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Photorhabdus luminescens
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: pTetLux1
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: join(1..3190, 10140..10220)
 - (D) OTHER INFORMATION: /standard_name= "vector pASK75"
/note= "Parts of plasmid originating from vector pASK75;
feature description below, SEQ ID NO: 9-11."
/citation= ([2])
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 3634..5082
 - (D) OTHER INFORMATION: /product= "Lux C"
/citation= ([1])
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 5097..6017
 - (D) OTHER INFORMATION: /product= "Lux D"
/citation= ([1])
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 6069..7148
 - (D) OTHER INFORMATION: /product= "Lux A"
/citation= ([1])
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 7166..8146
 - (D) OTHER INFORMATION: /product= "Lux B"
/citation= ([1])
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 8256..9437
 - (D) OTHER INFORMATION: /product= "Lux E"
/citation= ([1])
- (x) PUBLICATION INFORMATION:
 - (A) AUTHORS: Frackman,
 - (B) TITLE: Cloning, organization and expression of the
bioluminescence genes of Xenorhabdus
luminescens
 - (C) JOURNAL: J. Bacteriol.
 - (D) VOLUME: 172
 - (F) PAGES: 5767-5773
 - (G) DATE: 1990
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 3: FROM 3191 TO 10139

004240 29552550

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Skerra, A
 (B) TITLE: Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in Escherichia coli
 (C) JOURNAL: Gene
 (D) VOLUME: 151
 (E) ISSUE: 1-2
 (F) PAGES: 131-135
 (G) DATE: 30-DEC-1994
 (K) RELEVANT RESIDUES IN SEQ ID NO: 3: FROM 1 TO 3190

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```

AGCTTGACCT GTGAAGTGAA AAATGGCGCA CATTGTGCGA CATTTTTTTTT GTCTGCCGTT      60
TACCGCTACT GCGTCACGGA TCTCCACGCG CCCTGTAGCG GCGCATTAAAG CGCGGCGGGT      120
GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC      180
GCTTCTTCC CTTCCTTCT CGCCACGTTT GCCGGCTTTC CCCGTCAAGC TCTAAATCGG      240
GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT      300
TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTTCG CCCTTTGACG      360
TTGGAGTCCA CGTTCCTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCTT      420
ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA      480
AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAAC GCTTACAATT      540
TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA      600
CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA      660
AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTGCGCC TTATTCCTT TTTTGCGGCA      720
TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT      780
CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG      840
AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC      900
GCGGTATTAT CCCGTATTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT AACTATTCT      960
CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA     1020
GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA AACTGCGGC CAACTTACTT     1080
CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT     1140
GTAACGCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT     1200
GACACCACGA TGCCTGTAGC AATGGCAACA ACGTTGCGCA AACTATTAA TGGCGAACTA     1260
CTTACTCTAG CTTCCCGGCA ACAATTGATA GACTGGATGG AGGCGGATAA AGTTGCAGGA     1320
CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT     1380
GAGCGTGGCT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC     1440
GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT     1500
GAGATAGGTG CCTCACTGAT TAAGCATTGG TAGGAATTAA TGATGTCTCG TTTAGATAAA     1560

```

AGTAAAGTGA TTAACAGCGC ATTAGAGCTG CTTAATGAGG TCGGAATCGA AGGTTTAAACA 1620
 ACCCGTAAAC TCGCCCAGAA GCTAGGTGTA GAGCAGCCTA CATTGTATTG GCATGTAAAA 1680
 AATAAGCGGG CTTTGCTCGA CGCCTTAGCC ATTGAGATGT TAGATAGGCA CCATACTCAC 1740
 TTTTGCCCTT TAGAAGGGGA AAGCTGGCAA GATTTTITAC GTAATAACGC TAAAAGTTTT 1800
 AGATGTGCTT TACTAAGTCA TCGCGATGGA GCAAAAGTAC ATTTAGGTAC ACGGCCTACA 1860
 GAAAAACAGT ATGAACTCT CGAAAATCAA TTAGCCTTTT TATGCCAACA AGGTTTTTCA 1920
 CTAGAGAATG CATTATATGC ACTCAGCGCA GTGGGGCATT TTACTTTAGG TTGCGTATTG 1980
 GAAGATCAAG AGCATCAAGT CGCTAAAGAA GAAAGGGAAA CACCTACTAC TGATAGTATG 2040
 CCGCCATTAT TACGACAAGC TATCGAATTA TTTGATCACC AAGGTGCAGA GCCAGCCTTC 2100
 TTATTCGGCC TTGAATTGAT CATATGCGGA TTAGAAAAAC AACTTAAATG TGAAAGTGGG 2160
 TCTTAAAGC AGCATAACCT TTTTCCGTGA TGGTAACTTC ACTAGTTTAA AAGGATCTAG 2220
 GTGAAGATCC TTTTGTATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC 2280
 TGAGCGTCAG ACCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC 2340
 GTAATCTGCT GCTTGCAAAC AAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT 2400
 CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT 2460
 ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT 2520
 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT 2580
 CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG 2640
 GGGGGTTCTG GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA 2700
 CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG 2760
 GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 2820
 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC 2880
 TCGTCAGGGG GGCAGGAGCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT ACGGTTCTCTG 2940
 GCCTTTTGCT GGCCTTTTGC TCACATGACC CGACACCATC GAATGGCCAG ATGATTAATT 3000
 CCTAATTTTT GTTGACACTC TATCATTGAT AGAGTTATTT TACCACTCCC TATCAGTGAT 3060
 AGAGAAAAGT GAAATGAATA GTTCGACAAA AATCTAGATA ACGAGGGCAA AAAATGAAAA 3120
 AGACAGCTAT CGCGATTGCA GTGGCACTGG CTGGTTTCGC TACCGTAGCG CAGGCCTGAG 3180
 ACCAGAATTC TTCTTTAGAA ATCTGCCGGT AAAAATTAGA TTGCTATTCA ATCTATTTCT 3240
 ATCGGTATTT GTGAAATAAT ACTCAGGATA ATAATTTACA TAAATATTAT CACGCATTAG 3300
 AGAAGAGCAT GACTTTTTTA ATTTAACTT TTCATTAACA AATCTTGTTG ATATGAAAT 3360
 TTTCTTTGC TATTTTAAACA GATATTAAAA CGGGAATAGG CGTTATATTG ACGATCCATT 3420
 CAGTTAGATT AAAAACCTTG AGCAGAAAAT TTATATTATT ATCATAATTA TGACGAAAGT 3480
 TACAGGCCAG GAACCACGTA GTCAGAATCT GATTTTCTAT ATATTTGTTA TTTACATCGT 3540
 CATAACACAA AAATATAAGA AGCAAGTGTT GGTACGACCA GTTCGCAAGA TAGTTAAACA 3600

GCAACTTAAG TTGAAATTAC CCCCATTAATA TGG ATG GCA AAT ATG ACT AAA AAA	3654
Met Ala Asn Met Thr Lys Lys	555
ATT TCA TTC ATT ATT AAC GGC CAG GTT GAA ATC TTT CCC GAA AGT GAT	3702
Ile Ser Phe Ile Ile Asn Gly Gln Val Glu Ile Phe Pro Glu Ser Asp	560 565 570
GAT TTA GTG CAA TCC ATT AAT TTT GGT GAT AAT AGT GTT TAC CTG CCA	3750
Asp Leu Val Gln Ser Ile Asn Phe Gly Asp Asn Ser Val Tyr Leu Pro	575 580 585
ATA TTG AAT GAC TCT CAT GTA AAA AAC ATT ATT GAT TGT AAT GGA AAT	3798
Ile Leu Asn Asp Ser His Val Lys Asn Ile Ile Asp Cys Asn Gly Asn	590 595 600 605
AAC GAA TTA CGG TTG CAT AAC ATT GTC AAT TTT CTC TAT ACG GTA GGG	3846
Asn Glu Leu Arg Leu His Asn Ile Val Asn Phe Leu Tyr Thr Val Gly	610 615 620
CAA AGA TGG AAA AAT GAA GAA TAC TCA AGA CGC AGG ACA TAC ATT CGT	3894
Gln Arg Trp Lys Asn Glu Glu Tyr Ser Arg Arg Arg Thr Tyr Ile Arg	625 630 635
GAC TTA AAA AAA TAT ATG GGA TAT TCA GAA GAA ATG GCT AAG CTA GAG	3942
Asp Leu Lys Lys Tyr Met Gly Tyr Ser Glu Glu Met Ala Lys Leu Glu	640 645 650
GCC AAT TGG ATA TCT ATG ATT TTA TGT TCT AAA GGC GGC CTT TAT GAT	3990
Ala Asn Trp Ile Ser Met Ile Leu Cys Ser Lys Gly Gly Leu Tyr Asp	655 660 665
GTT GTA GAA AAT GAA CTT GGT TCT CGC CAT ATC ATG GAT GAA TGG CTA	4038
Val Val Glu Asn Glu Leu Gly Ser Arg His Ile Met Asp Glu Trp Leu	670 675 680 685
CCT CAG GAT GAA AGT TAT GTT CGG GCT TTT CCG AAA GGT AAA TCT GTA	4086
Pro Gln Asp Glu Ser Tyr Val Arg Ala Phe Pro Lys Gly Lys Ser Val	690 695 700
CAT CTG TTG GCA GGT AAT GTT CCA TTA TCT GGG ATC ATG TCT ATA TTA	4134
His Leu Leu Ala Gly Asn Val Pro Leu Ser Gly Ile Met Ser Ile Leu	705 710 715
CGC GCA ATT TTA ACT AAG AAT CAG TGT ATT ATA AAA ACA TCG TCA ACC	4182
Arg Ala Ile Leu Thr Lys Asn Gln Cys Ile Ile Lys Thr Ser Ser Thr	720 725 730
GAT CCT TTT ACC GCT AAT GCA TTA GCG TTA AGT TTT ATT GAT GTA GAC	4230
Asp Pro Phe Thr Ala Asn Ala Leu Ala Leu Ser Phe Ile Asp Val Asp	735 740 745
CCT AAT CAT CCG ATA ACG CGC TCT TTA TCT GTT ATA TAT TGG CCC CAC	4278
Pro Asn His Pro Ile Thr Arg Ser Leu Ser Val Ile Tyr Trp Pro His	750 755 760 765
CAA GGT GAT ACA TCA CTC GCA AAA GAA ATT ATG CGA CAT GCG GAT GTT	4326
Gln Gly Asp Thr Ser Leu Ala Lys Glu Ile Met Arg His Ala Asp Val	770 775 780
ATT GTC GCT TGG GGA GGG CCA GAT GCG ATT AAT TGG GCG GTA GAG CAT	4374
Ile Val Ala Trp Gly Gly Pro Asp Ala Ile Asn Trp Ala Val Glu His	785 790 795
GCG CCA TCT TAT GCT GAT GTG ATT AAA TTT GGT TCT AAA AAG AGT CTT	4422
Ala Pro Ser Tyr Ala Asp Val Ile Lys Phe Gly Ser Lys Lys Ser Leu	800 805 810

TGC ATT ATC GAT AAT CCT GTT GAT TTG ACG TCC GCA GCG ACA GGT GCG Cys Ile Ile Asp Asn Pro Val Asp Leu Thr Ser Ala Ala Thr Gly Ala 815 820 825	4470
GCT CAT GAT GTT TGT TTT TAC GAT CAG CGA GCT TGT TTT TCT GCC CAA Ala His Asp Val Cys Phe Tyr Asp Gln Arg Ala Cys Phe Ser Ala Gln 830 835 840 845	4518
AAC ATA TAT TAC ATG GGA AAT CAT TAT GAG GAA TTT AAG TTA GCG TTG Asn Ile Tyr Tyr Met Gly Asn His Tyr Glu Glu Phe Lys Leu Ala Leu 850 855 860	4566
ATA GAA AAA CTT AAT CTA TAT GCG CAT ATA TTA CCG AAT GCC AAA AAA Ile Glu Lys Leu Asn Leu Tyr Ala His Ile Leu Pro Asn Ala Lys Lys 865 870 875	4614
GAT TTT GAT GAA AAG GCG GCC TAT TCT TTA GTT CAA AAA GAA AGC TTG Asp Phe Asp Glu Lys Ala Ala Tyr Ser Leu Val Gln Lys Glu Ser Leu 880 885 890	4662
TTT GCT GGA TTA AAA GTA GAG GTG GAT ATT CAT CAA CGT TGG ATG ATT Phe Ala Gly Leu Lys Val Glu Val Asp Ile His Gln Arg Trp Met Ile 895 900 905	4710
ATT GAG TCA AAT GCA GGT GTG GAA TTT AAT CAA CCA CTT GGC AGA TGT Ile Glu Ser Asn Ala Gly Val Glu Phe Asn Gln Pro Leu Gly Arg Cys 910 915 920 925	4758
GTG TAC CTT CAT CAC GTC GAT AAT ATT GAG CAA ATA TTG CCT TAT GTT Val Tyr Leu His His Val Asp Asn Ile Glu Gln Ile Leu Pro Tyr Val 930 935 940	4806
CAA AAA AAT AAG ACG CAA ACC ATA TCT ATT TTT CCT TGG GAG TCA TCA Gln Lys Asn Lys Thr Gln Thr Ile Ser Ile Phe Pro Trp Glu Ser Ser 945 950 955	4854
TTT AAA TAT CGA GAT GCG TTA GCA TTA AAA GGT GCG GAA AGG ATT GTA Phe Lys Tyr Arg Asp Ala Leu Ala Leu Lys Gly Ala Glu Arg Ile Val 960 965 970	4902
GAA GCA GGA ATG AAT AAC ATA TTT CGA GTT GGT GGA TCT CAT GAC GGA Glu Ala Gly Met Asn Asn Ile Phe Arg Val Gly Gly Ser His Asp Gly 975 980 985	4950
ATG AGA CCG TTG CAA CGA TTA GTG ACA TAT ATT TCT CAT GAA AGG CCA Met Arg Pro Leu Gln Arg Leu Val Thr Tyr Ile Ser His Glu Arg Pro 990 995 1000 1005	4998
TCT AAC TAT ACG GCT AAG GAT GTT GCG GTT GAA ATA GAA CAG ACT CGA Ser Asn Tyr Thr Ala Lys Asp Val Ala Val Glu Ile Glu Gln Thr Arg 1010 1015 1020	5046
TTC CTG GAA GAA GAT AAG TTC CTT GTA TTT GTC CCA TAATAGGTAA Phe Leu Glu Glu Asp Lys Phe Leu Val Phe Val Pro 1025 1030	5092
AAGT ATG GAA AAT GAA TCA AAA TAT AAA ACC ATC GAC CAC GTT ATT TGT Met Glu Asn Glu Ser Lys Tyr Lys Thr Ile Asp His Val Ile Cys 1 5 10 15	5141
GTT GAA GGA AAT AAA AAA ATT CAT GTT TGG GAA ACG CTG CCA GAA GAA Val Glu Gly Asn Lys Lys Ile His Val Trp Glu Thr Leu Pro Glu Glu 20 25 30	5189
AAC AGC CCA AAG AGA AAG AAT GCC ATT ATT ATT GCG TCT GGT TTT GCC Asn Ser Pro Lys Arg Lys Asn Ala Ile Ile Ala Ser Gly Phe Ala 35 40 45	5237

CGC AGG ATG GAT CAT TTT GCT GGT CTG GCG GAA TAT TTA TCG CGG AAT	5285
Arg Arg Met Asp His Phe Ala Gly Leu Ala Glu Tyr Leu Ser Arg Asn	
50 55 60	
GGA TTT CAT GTG ATC CGC TAT GAT TCG CTT CAC CAC GTT GGA TTG AGT	5333
Gly Phe His Val Ile Arg Tyr Asp Ser Leu His Val Gly Leu Ser	
65 70 75	
TCA GGG ACA ATT GAT GAA TTT ACA ATG TCT ATA GGA AAG CAG AGC TTG	5381
Ser Gly Thr Ile Asp Glu Phe Thr Met Ser Ile Gly Lys Gln Ser Leu	
80 85 90 95	
TTA GCA GTG GTT GAT TGG TTA ACT ACA CGA AAA ATA AAT AAC TTC GGT	5429
Leu Ala Val Val Asp Trp Leu Thr Thr Arg Lys Ile Asn Asn Phe Gly	
100 105 110	
ATG TTG GCT TCA AGC TTA TCT GCG CGG ATA GCT TAT GCA AGC CTA TCT	5477
Met Leu Ala Ser Ser Leu Ser Ala Arg Ile Ala Tyr Ala Ser Leu Ser	
115 120 125	
GAA ATC AAT GCT TCG TTT TTA ATC ACC GCA GTC GGT GTT GTT AAC TTA	5525
Glu Ile Asn Ala Ser Phe Leu Ile Thr Ala Val Gly Val Val Asn Leu	
130 135 140	
AGA TAT TCT CTT GAA AGA GCT TTA GGG TTT GAT TAT CTC AGT CTA CCC	5573
Arg Tyr Ser Leu Glu Arg Ala Leu Gly Phe Asp Tyr Leu Ser Leu Pro	
145 150 155	
ATT AAT GAA TTG CCG GAT AAT CTA GAT TTT GAA GGC CAT AAA TTG GGT	5621
Ile Asn Glu Leu Pro Asp Asn Leu Asp Phe Glu Gly His Lys Leu Gly	
160 165 170 175	
GCT GAA GTC TTT GCG AGA GAT TGT CTT GAT TTT GGT TGG GAA GAT TTA	5669
Ala Glu Val Phe Ala Arg Asp Cys Leu Asp Phe Gly Trp Glu Asp Leu	
180 185 190	
GCT TCT ACA ATT AAT AAC ATG ATG TAT CTT GAT ATA CCG TTT ATT GCT	5717
Ala Ser Thr Ile Asn Asn Met Met Tyr Leu Asp Ile Pro Phe Ile Ala	
195 200 205	
TTT ACT GCA AAT AAC GAT AAT TGG GTC AAG CAA GAT GAA GTT ATC ACA	5765
Phe Thr Ala Asn Asn Asp Asn Trp Val Lys Gln Asp Glu Val Ile Thr	
210 215 220	
TTG TTA TCA AAT ATT CGT AGT AAT CGA TGC AAG ATA TAT TCT TTG TTA	5813
Leu Leu Ser Asn Ile Arg Ser Asn Arg Cys Lys Ile Tyr Ser Leu Leu	
225 230 235	
GGA AGT TCG CAT GAC TTG AGT GAA AAT TTA GTG GTC CTG CGC AAT TTT	5861
Gly Ser Ser His Asp Leu Ser Glu Asn Leu Val Val Leu Arg Asn Phe	
240 245 250 255	
TAT CAA TCG GTT ACG AAA GCC GCT ATC GCG ATG GAT AAT GAT CAT CTG	5909
Tyr Gln Ser Val Thr Lys Ala Ala Ile Ala Met Asp Asn Asp His Leu	
260 265 270	
GAT ATT GAT GTT GAT ATT ACT GAA CCG TCA TTT GAA CAT TTA ACT ATT	5957
Asp Ile Asp Val Asp Ile Thr Glu Pro Ser Phe Glu His Leu Thr Ile	
275 280 285	
GCG ACA GTC AAT GAA CGC CGA ATG AGA ATT GAG ATT GAA AAT CAA GCA	6005
Ala Thr Val Asn Glu Arg Arg Met Arg Ile Glu Ile Glu Asn Gln Ala	
290 295 300	
ATT TCT CTG TCT TAAATCTAT TGAGATATTC TATCACTCAA ATAGCAATAT	6057
Ile Ser Leu Ser	
305	

AAGGACTCTC	T	ATG	AAA	TTT	GGA	AAC	TTT	TTG	CTT	ACA	TAC	CAA	CCT	CCC	6107
		Met	Lys	Phe	Gly	Asn	Phe	Leu	Leu	Thr	Tyr	Gln	Pro	Pro	
		1				5					10				
CAA	TTT	TCT	CAA	ACA	GAG	GTA	ATG	AAA	CGT	TTG	GTT	AAA	TTA	GGT	CGC
Gln	Phe	Ser	Gln	Thr	Glu	Val	Met	Lys	Arg	Leu	Val	Lys	Leu	Gly	Arg
	15					20					25				6155
ATC	TCT	GAG	GAG	TGT	GGT	TTT	GAT	ACC	GTA	TGG	TTA	CTG	GAG	CAT	CAT
Ile	Ser	Glu	Glu	Cys	Gly	Phe	Asp	Thr	Val	Trp	Leu	Leu	Glu	His	His
	30				35					40				45	6203
TTC	ACG	GAG	TTT	GGT	TTG	CTT	GGT	AAC	CCT	TAT	GTC	GCT	GCT	GCA	TAT
Phe	Thr	Glu	Phe	Gly	Leu	Leu	Gly	Asn	Pro	Tyr	Val	Ala	Ala	Ala	Tyr
				50				55						60	6251
TTA	CTT	GCG	GCG	ACT	AAA	AAA	TTG	AAT	GTA	GGA	ACT	GCC	GCT	ATT	GTT
Leu	Leu	Gly	Ala	Thr	Lys	Lys	Leu	Asn	Val	Gly	Thr	Ala	Ala	Ile	Val
			65				70					75			6299
CTT	CCC	ACA	GCC	CAT	CCA	GTA	CGC	CAA	CTT	GAA	GAT	GTG	AAT	TTA	TTG
Leu	Pro	Thr	Ala	His	Pro	Val	Arg	Gln	Leu	Glu	Asp	Val	Asn	Leu	Leu
		80				85						90			6347
GAT	CAA	ATG	TCA	AAA	GGA	CGA	TTT	CGG	TTT	GGT	ATT	TGC	CGA	GGG	CTT
Asp	Gln	Met	Ser	Lys	Gly	Arg	Phe	Arg	Phe	Gly	Ile	Cys	Arg	Gly	Leu
	95					100					105				6395
TAC	AAC	AAG	GAC	TTT	CGC	GTA	TTC	GGC	ACA	GAT	ATG	AAT	AAC	AGT	CGC
Tyr	Asn	Lys	Asp	Phe	Arg	Val	Phe	Gly	Thr	Asp	Met	Asn	Asn	Ser	Arg
	110				115					120					6443
GCC	TTA	GCG	GAA	TGC	TGG	TAC	GGG	CTG	ATA	AAG	AAT	GCG	ATG	ACA	GAG
Ala	Leu	Ala	Glu	Cys	Trp	Tyr	Gly	Leu	Ile	Lys	Asn	Gly	Met	Thr	Glu
				130				135						140	6491
GGA	TAT	ATG	GAA	GCT	GAT	AAT	GAA	CAT	ATC	AAG	TTC	CAT	AAG	GTA	AAA
Gly	Tyr	Met	Glu	Ala	Asp	Asn	Glu	His	Ile	Lys	Phe	His	Lys	Val	Lys
			145				150						155		6539
GTA	AAC	CCC	GCG	GCG	TAT	AGC	AGA	GGT	GGC	GCA	CCG	GTT	TAT	GTG	GTG
Val	Asn	Pro	Ala	Ala	Tyr	Ser	Arg	Gly	Gly	Ala	Pro	Val	Tyr	Val	Val
		160					165					170			6587
GCT	GAA	TCA	GCT	TCG	ACG	ACT	GAG	TGG	GCT	GCT	CAA	TTT	GGC	CTA	CCG
Ala	Glu	Ser	Ala	Ser	Thr	Thr	Glu	Trp	Ala	Ala	Gln	Phe	Gly	Leu	Pro
	175					180					185				6635
ATG	ATA	TTA	AGT	TGG	ATT	ATA	AAT	ACT	AAC	GAA	AAG	AAA	GCA	CAA	CTT
Met	Ile	Leu	Ser	Trp	Ile	Ile	Asn	Thr	Asn	Glu	Lys	Lys	Ala	Gln	Leu
	190				195					200					205
GAG	CTT	TAT	AAT	GAA	GTG	GCT	CAA	GAA	TAT	GGG	CAC	GAT	ATT	CAT	AAT
Glu	Leu	Tyr	Asn	Glu	Val	Ala	Gln	Glu	Tyr	Gly	His	Asp	Ile	His	Asn
				210					215					220	6731
ATC	GAC	CAT	TGC	TTA	TCA	TAT	ATA	ACA	TCT	GTA	GAT	CAT	GAC	TCA	ATT
Ile	Asp	His	Cys	Leu	Ser	Tyr	Ile	Thr	Ser	Val	Asp	His	Asp	Ser	Ile
			225					230					235		6779
AAA	GCG	AAA	GAG	ATT	TGC	CGG	AAA	TTT	CTG	GGG	CAT	TGG	TAT	GAT	TCT
Lys	Ala	Lys	Glu	Ile	Cys	Arg	Lys	Phe	Leu	Gly	His	Trp	Tyr	Asp	Ser
		240					245					250			6827
TAT	GTG	AAT	GCT	ACG	ACT	ATT	TTT	GAT	GAT	TCA	GAC	CAA	ACA	AGA	GGT
Tyr	Val	Asn	Ala	Thr	Thr	Ile	Phe	Asp	Asp	Ser	Asp	Gln	Thr	Arg	Gly
	255					260					265				6875

TAT GAT TTC AAT AAA GGG CAG TGG CGT GAC TTT GTA TTA AAA GGA CAT Tyr Asp Phe Asn Lys Gly Gln Trp Arg Asp Phe Val Leu Lys Gly His 270 275 280 285	6923
AAA GAT ACT AAT CGC CGT ATT GAT TAC AGT TAC GAA ATC AAT CCC GTG Lys Asp Thr Asn Arg Arg Ile Asp Tyr Ser Tyr Glu Ile Asn Pro Val 290 295 300	6971
GGA ACG CCG CAG GAA TGT ATT GAC ATA ATT CAA AAA GAC ATT GAT GCT Gly Thr Pro Gln Glu Cys Ile Asp Ile Ile Gln Lys Asp Ile Asp Ala 305 310 315	7019
ACA GGA ATA TCA AAT ATT TGT TGT GGA TTT GAA GCT AAT GGA ACA GTA Thr Gly Ile Ser Asn Ile Cys Cys Gly Phe Glu Ala Asn Gly Thr Val 320 325 330	7067
GAC GAA ATT ATT GCT TCC ATG AAG CTC TTC CAG TCT GAT GTC ATG CCA Asp Glu Ile Ile Ala Ser Met Lys Leu Phe Gln Ser Asp Val Met Pro 335 340 345	7115
TTT CTT AAA GAA AAA CAA CGT TCG CTA TTA TAT TAGCTAAGGA GAAAGAA Phe Leu Lys Glu Lys Gln Arg Ser Leu Leu Tyr 350 355 360	7165
ATG AAA TTT GGA TTG TTC TTC CTT AAC TTC ATC AAT TCA ACA ACT GTT Met Lys Phe Gly Leu Phe Phe Leu Asn Phe Ile Asn Ser Thr Thr Val 1 5 10 15	7213
CAA GAA CAA AGT ATA GTT CGC ATG CAG GAA ATA ACG GAG TAT GTT GAT Gln Glu Gln Ser Ile Val Arg Met Gln Glu Ile Thr Glu Tyr Val Asp 20 25 30	7261
AAG TTG AAT TTT GAA CAG ATT TTA GTG TAT GAA AAT CAT TTT TCA GAT Lys Leu Asn Phe Glu Gln Ile Leu Val Tyr Glu Asn His Phe Ser Asp 35 40 45	7309
AAT GGT GTT GTC GGC GCT CCT CTG ACT GTT TCT GGT TTT CTG CTC GGT Asn Gly Val Val Gly Ala Pro Leu Thr Val Ser Gly Phe Leu Leu Gly 50 55 60	7357
TTA ACA GAG AAA ATT AAA ATT GGT TCA TTA AAT CAC ATC ATT ACA ACT Leu Thr Glu Lys Ile Lys Ile Gly Ser Leu Asn His Ile Ile Thr Thr 65 70 75 80	7405
CAT CAT CCT GTC GCC ATA GCG GAG GAA GCT TGC TTA TTG GAT CAG TTA His His Pro Val Ala Ile Ala Glu Glu Ala Cys Leu Leu Asp Gln Leu 85 90 95	7453
AGT GAA GGG AGA TTT ATT TTA GGG TTT AGT GAT TGC GAA AAA AAA GAT Ser Glu Gly Arg Phe Ile Leu Gly Phe Ser Asp Cys Glu Lys Lys Asp 100 105 110	7501
GAA ATG CAT TTT TTT AAT CGC CCG GTT GAA TAT CAA CAG CAA CTA TTT Glu Met His Phe Phe Asn Arg Pro Val Glu Tyr Gln Gln Gln Leu Phe 115 120 125	7549
GAA GAG TGT TAT GAA ATC ATT AAC GAT GCT TTA ACA ACA GGC TAT TGT Glu Glu Cys Tyr Glu Ile Ile Asn Asp Ala Leu Thr Thr Gly Tyr Cys 130 135 140	7597
AAT CCA GAT AAC GAT TTT TAT AGC TTC CCT AAA ATA TCT GTA AAT CCC Asn Pro Asp Asn Asp Phe Tyr Ser Phe Pro Lys Ile Ser Val Asn Pro 145 150 155 160	7645
CAT GCT TAT ACG CCA GGC GGA CCT CGG AAA TAT GTA ACA GCA ACC AGT His Ala Tyr Thr Pro Gly Gly Pro Arg Lys Tyr Val Thr Ala Thr Ser 165 170 175	7693

CAT CAT ATT GTT GAG TGG GCG GCC AAA AAA GGT ATT CCT CTC ATC TTT His His Ile Val Glu Trp Ala Ala Lys Lys Gly Ile Pro Leu Ile Phe 180 185 190	7741
AAG TGG GAT GAT TCT AAT GAT GTT AGA TAT GAA TAT GCT GAA AGA TAT Lys Trp Asp Asp Ser Asn Asp Val Arg Tyr Glu Tyr Ala Glu Arg Tyr 195 200 205	7789
AAA GCC GTT GCG GAT AAA TAT GAC GTT GAC CTA TCA GAG ATA GAC CAT Lys Ala Val Ala Asp Lys Tyr Asp Val Asp Leu Ser Glu Ile Asp His 210 215 220	7837
CAG TTA ATG ATA TTA GTT AAC TAT AAC GAA GAT AGT AAT AAA GCT AAA Gln Leu Met Ile Leu Val Asn Tyr Asn Glu Asp Ser Asn Lys Ala Lys 225 230 235 240	7885
CAA GAG ACG CGT GCA TTT ATT AGT GAT TAT GTT CTT GAA ATG CAC CCT Gln Glu Thr Arg Ala Phe Ile Ser Asp Tyr Val Leu Glu Met His Pro 245 250 255	7933
AAT GAA AAT TTC GAA AAT AAA CTT GAA GAA ATA ATT GCA GAA AAC GCT Asn Glu Asn Phe Glu Asn Lys Leu Glu Glu Ile Ile Ala Glu Asn Ala 260 265 270	7981
GTC GGA AAT TAT ACG GAG TGT ATA ACT GCG GCT AAG TTG GCA ATT GAA Val Gly Asn Tyr Thr Glu Cys Ile Thr Ala Ala Lys Leu Ala Ile Glu 275 280 285	8029
AAG TGT GGT GCG AAA AGT GTA TTG CTG TCC TTT GAA CCA ATG AAT GAT Lys Cys Gly Ala Lys Ser Val Leu Leu Ser Phe Glu Pro Met Asn Asp 290 295 300	8077
TTG ATG AGC CAA AAA AAT GTA ATC AAT ATT GTT GAT GAT AAT ATT AAG Leu Met Ser Gln Lys Asn Val Ile Asn Ile Val Asp Asp Asn Ile Lys 305 310 315 320	8125
AAG TAC CAC ATG GAA TAT ACC TAATAGATTT CGAGTTGCAG CGAGGCGGCA Lys Tyr His Met Glu Tyr Thr 325	8176
AGTGAACGAA TCCCCAGGAG CATAGATAAC TATGTGACTG GGGTGAGTGA AAGCAGCCAA	8236
CAAAGCAGCA GCTTGAAAG ATG AAG GGT ATA AAA GAG TAT GAC AGC AGT GCT Met Lys Gly Ile Lys Glu Tyr Asp Ser Ser Ala 1 5 10	8288
GCC ATA CTT TCT AAT ATT ATC TTG AGG AGT AAA ACA GGT ATG ACT TCA Ala Ile Leu Ser Asn Ile Ile Leu Arg Ser Lys Thr Gly Met Thr Ser 15 20 25	8336
TAT GTT GAT AAA CAA GAA ATT ACA GCA AGC TCA GAA ATT GAT GAT TTG Tyr Val Asp Lys Gln Glu Ile Thr Ala Ser Ser Glu Ile Asp Asp Leu 30 35 40	8384
ATT TTT TCG AGC GAT CCA TTA GTG TGG TCT TAC GAC GAG CAG GAA AAA Ile Phe Ser Ser Asp Pro Leu Val Trp Ser Tyr Asp Glu Gln Glu Lys 45 50 55	8432
ATC AGA AAG AAA CTT GTG CTT GAT GCA TTT CGT AAT CAT TAT AAA CAT Ile Arg Lys Lys Leu Val Leu Asp Ala Phe Arg Asn His Tyr Lys His 60 65 70 75	8480
TGT CGA GAA TAT CGT CAC TAC TGT CAG GCA CAC AAA GTA GAT GAC AAT Cys Arg Glu Tyr Arg His Tyr Cys Gln Ala His Lys Val Asp Asp Asn 80 85 90	8528

ATT ACG GAA ATT GAT GAC ATA CCT GTA TTC CCA ACA TCG GTT TTT AAG Ile Thr Glu Ile Asp Asp Ile Pro Val Phe Pro Thr Ser Val Phe Lys 95 100 105	8576
TTT ACT CGC TTA TTA ACT TCT CAG GAA AAC GAG ATT GAA AGT TGG TTT Phe Thr Arg Leu Leu Thr Ser Gln Glu Asn Glu Ile Glu Ser Trp Phe 110 115 120	8624
ACC AGT AGC GGC ACG AAT GGT TTA AAA AGT CAG GTG GCG CGT GAC AGA Thr Ser Ser Gly Thr Asn Gly Leu Lys Ser Gln Val Ala Arg Asp Arg 125 130 135	8672
TTA AGT ATT GAG AGA CTC TTA GGC TCT GTG AGT TAT GGC ATG AAA TAT Leu Ser Ile Glu Arg Leu Leu Gly Ser Val Ser Tyr Gly Met Lys Tyr 140 145 150 155	8720
GTT GGT AGT TGG TTT GAT CAT CAA ATA GAA TTA GTC AAT TTG GGA CCA Val Gly Ser Trp Phe Asp His Gln Ile Glu Leu Val Asn Leu Gly Pro 160 165 170	8768
GAT AGA TTT AAT GCT CAT AAT ATT TGG TTT AAA TAT GTT ATG AGT TTG Asp Arg Phe Asn Ala His Asn Ile Trp Phe Lys Tyr Val Met Ser Leu 175 180 185	8816
GTG GAA TTG TTA TAT CCT ACG ACA TTT ACC GTA ACA GAA GAA CGA ATA Val Glu Leu Leu Tyr Pro Thr Thr Phe Thr Val Thr Glu Glu Arg Ile 190 195 200	8864
GAT TTT GTT AAA ACA TTG AAT AGT CTT GAA CGA ATA AAA AAT CAA GGG Asp Phe Val Lys Thr Leu Asn Ser Leu Glu Arg Ile Lys Asn Gln Gly 205 210 215	8912
AAA GAT CTT TGT CTT ATT GGT TCG CCA TAC TTT ATT TAT TTA CTC TGC Lys Asp Leu Cys Leu Ile Gly Ser Pro Tyr Phe Ile Tyr Leu Leu Cys 220 225 230 235	8960
CAT TAT ATG AAA GAT AAA AAA ATC TCA TTT TCT GGA GAT AAA AGC CTT His Tyr Met Lys Asp Lys Lys Ile Ser Phe Ser Gly Asp Lys Ser Leu 240 245 250	9008
TAT ATC ATA ACC GGA GGC GGC TGG AAA AGT TAC GAA AAA GAA TCT CTG Tyr Ile Ile Thr Gly Gly Gly Trp Lys Ser Tyr Glu Lys Glu Ser Leu 255 260 265	9056
AAA CGT GAT GAT TTC AAT CAT CTT TTA TTT GAT ACT TTC AAT CTC AGT Lys Arg Asp Asp Phe Asn His Leu Leu Phe Asp Thr Phe Asn Leu Ser 270 275 280	9104
GAT ATT AGT CAG ATC CGA GAT ATA TTT AAT CAA GTT GAA CTC AAC ACT Asp Ile Ser Gln Ile Arg Asp Ile Phe Asn Gln Val Glu Leu Asn Thr 285 290 295	9152
TGT TTC TTT GAG GAT GAA ATG CAG CGT AAA CAT GTT CCG CCG TGG GTA Cys Phe Phe Glu Asp Glu Met Gln Arg Lys His Val Pro Pro Trp Val 300 305 310 315	9200
TAT GCG CGA GCG CTT GAT CCT GAA ACG TTG AAA CCT GTA CCT GAT GGA Tyr Ala Arg Ala Leu Asp Pro Glu Thr Leu Lys Pro Val Pro Asp Gly 320 325 330	9248
ACG CCG GGG TTG ATG AGT TAT ATG GAT GCG TCA GCA ACC AGT TAT CCA Thr Pro Gly Leu Met Ser Tyr Met Asp Ala Ser Ala Thr Ser Tyr Pro 335 340 345	9296
GCA TTT ATT GTT ACC GAT GAT GTC GGG ATA ATT AGC AGA GAA TAT GGT Ala Phe Ile Val Thr Asp Asp Val Gly Ile Ile Ser Arg Glu Tyr Gly 350 355 360	9344

AAG TAT CCC GGC GTG CTC GTT GAA ATT TTA CGT CGC GTC AAT ACG AGG 9392
 Lys Tyr Pro Gly Val Leu Val Glu Ile Leu Arg Arg Val Asn Thr Arg
 365 370 375
 ACG CAG AAA GGG TGT GCT TTA AGC TTA ACC GAA GCG TTT GAT AGT 9437
 Thr Gln Lys Gly Cys Ala Leu Ser Leu Thr Glu Ala Phe Asp Ser
 380 385 390
 TGATATCCTT TGCCTAATTG TAAGTGAAT GCTTGCCTTA TATAAATCTG AATGACATCT 9497
 ACACTTTACA AAATTCTCCA AAACATCCAC ATTTGGGTAC TTGATAGAGG TTTATGGGGT 9557
 TGGCTTAACA TTGTTCTCAT TGTATTATT GGCTCAAAGC AAAAGGAGAT AACATGAAAA 9617
 AATTGGCAGT TATGCTTGCA TTGGGAATGA TTAGCTTTGG TGCAATGGCA GTTGATGGGT 9677
 ATAAAGATGC AAAGTTTGGC ATGACAGAAG AAGAGTTTCT TTCGAAGAGG TTATGTGATT 9737
 TTGAAAAATT TGAGGGAGAT TCTCGAATAG AAGAAGTATC ACTTTATTCA TGTTCCTGACT 9797
 TTTCGTTTGC TAACAAAAAG CGTGAAGCAA TGGCATTTTT TTTAAATGGG AAATTTAAAA 9857
 GATTAGAGAT TAATATTGGC AGACTTGTGA AGCCAGTAAG CAAATCGTTA ACGAAAAAGT 9917
 ACGGAGATGG ATCATCGTAT CCATCAAAAG AAGAATTGTA GAACGCGCTA AAATACAATG 9977
 GAACTATGTC TATAGGTTAT GATAATAATA CGGTATTAGT TGATATACAT ATAATATGTG 10037
 GCAAAGAAGG CATAGAAACC AGTCAACTGA TTTATACGAG TCCAGATGTT TATACGCTCC 10097
 CAGATTTTCG AGAAAAAATC CAGGAATTAA AGGGATTAA GGAATTCGAG CTCGGTACCC 10157
 GGGGATCCCT CGAGGTCGAC CTGCAGGCAG CGCTTGGCGT CACCCGCAGT TCGGTGGTTA 10217
 ATA 10220

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 483 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ala Asn Met Thr Lys Lys Ile Ser Phe Ile Ile Asn Gly Gln Val
 1 5 10 15
 Glu Ile Phe Pro Glu Ser Asp Asp Leu Val Gln Ser Ile Asn Phe Gly
 20 25 30
 Asp Asn Ser Val Tyr Leu Pro Ile Leu Asn Asp Ser His Val Lys Asn
 35 40 45
 Ile Ile Asp Cys Asn Gly Asn Asn Glu Leu Arg Leu His Asn Ile Val
 50 55 60
 Asn Phe Leu Tyr Thr Val Gly Gln Arg Trp Lys Asn Glu Glu Tyr Ser
 65 70 75 80
 Arg Arg Arg Thr Tyr Ile Arg Asp Leu Lys Lys Tyr Met Gly Tyr Ser
 85 90 95
 Glu Glu Met Ala Lys Leu Glu Ala Asn Trp Ile Ser Met Ile Leu Cys
 100 105 110

Ser Lys Gly Gly Leu Tyr Asp Val Val Glu Asn Glu Leu Gly Ser Arg
 115 120 125
 His Ile Met Asp Glu Trp Leu Pro Gln Asp Glu Ser Tyr Val Arg Ala
 130 135 140
 Phe Pro Lys Gly Lys Ser Val His Leu Leu Ala Gly Asn Val Pro Leu
 145 150 155 160
 Ser Gly Ile Met Ser Ile Leu Arg Ala Ile Leu Thr Lys Asn Gln Cys
 165 170 175
 Ile Ile Lys Thr Ser Ser Thr Asp Pro Phe Thr Ala Asn Ala Leu Ala
 180 185 190
 Leu Ser Phe Ile Asp Val Asp Pro Asn His Pro Ile Thr Arg Ser Leu
 195 200 205
 Ser Val Ile Tyr Trp Pro His Gln Gly Asp Thr Ser Leu Ala Lys Glu
 210 215 220
 Ile Met Arg His Ala Asp Val Ile Val Ala Trp Gly Gly Pro Asp Ala
 225 230 235 240
 Ile Asn Trp Ala Val Glu His Ala Pro Ser Tyr Ala Asp Val Ile Lys
 245 250 255
 Phe Gly Ser Lys Lys Ser Leu Cys Ile Ile Asp Asn Pro Val Asp Leu
 260 265 270
 Thr Ser Ala Ala Thr Gly Ala Ala His Asp Val Cys Phe Tyr Asp Gln
 275 280 285
 Arg Ala Cys Phe Ser Ala Gln Asn Ile Tyr Tyr Met Gly Asn His Tyr
 290 295 300
 Glu Glu Phe Lys Leu Ala Leu Ile Glu Lys Leu Asn Leu Tyr Ala His
 305 310 315 320
 Ile Leu Pro Asn Ala Lys Lys Asp Phe Asp Glu Lys Ala Ala Tyr Ser
 325 330 335
 Leu Val Gln Lys Glu Ser Leu Phe Ala Gly Leu Lys Val Glu Val Asp
 340 345 350
 Ile His Gln Arg Trp Met Ile Ile Glu Ser Asn Ala Gly Val Glu Phe
 355 360 365
 Asn Gln Pro Leu Gly Arg Cys Val Tyr Leu His His Val Asp Asn Ile
 370 375 380
 Glu Gln Ile Leu Pro Tyr Val Gln Lys Asn Lys Thr Gln Thr Ile Ser
 385 390 395 400
 Ile Phe Pro Trp Glu Ser Ser Phe Lys Tyr Arg Asp Ala Leu Ala Leu
 405 410 415
 Lys Gly Ala Glu Arg Ile Val Glu Ala Gly Met Asn Asn Ile Phe Arg
 420 425 430
 Val Gly Gly Ser His Asp Gly Met Arg Pro Leu Gln Arg Leu Val Thr
 435 440 445
 Tyr Ile Ser His Glu Arg Pro Ser Asn Tyr Thr Ala Lys Asp Val Ala
 450 455 460
 Val Glu Ile Glu Gln Thr Arg Phe Leu Glu Glu Asp Lys Phe Leu Val
 465 470 475 480

Phe Val Pro

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 307 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```

Met Glu Asn Glu Ser Lys Tyr Lys Thr Ile Asp His Val Ile Cys Val
 1          5          10          15
Glu Gly Asn Lys Lys Ile His Val Trp Glu Thr Leu Pro Glu Glu Asn
      20          25          30
Ser Pro Lys Arg Lys Asn Ala Ile Ile Ile Ala Ser Gly Phe Ala Arg
      35          40          45
Arg Met Asp His Phe Ala Gly Leu Ala Glu Tyr Leu Ser Arg Asn Gly
      50          55          60
Phe His Val Ile Arg Tyr Asp Ser Leu His His Val Gly Leu Ser Ser
      65          70          75          80
Gly Thr Ile Asp Glu Phe Thr Met Ser Ile Gly Lys Gln Ser Leu Leu
      85          90          95
Ala Val Val Asp Trp Leu Thr Thr Arg Lys Ile Asn Asn Phe Gly Met
      100          105          110
Leu Ala Ser Ser Leu Ser Ala Arg Ile Ala Tyr Ala Ser Leu Ser Glu
      115          120          125
Ile Asn Ala Ser Phe Leu Ile Thr Ala Val Gly Val Val Asn Leu Arg
      130          135          140
Tyr Ser Leu Glu Arg Ala Leu Gly Phe Asp Tyr Leu Ser Leu Pro Ile
      145          150          155          160
Asn Glu Leu Pro Asp Asn Leu Asp Phe Glu Gly His Lys Leu Gly Ala
      165          170          175
Glu Val Phe Ala Arg Asp Cys Leu Asp Phe Gly Trp Glu Asp Leu Ala
      180          185          190
Ser Thr Ile Asn Asn Met Met Tyr Leu Asp Ile Pro Phe Ile Ala Phe
      195          200          205
Thr Ala Asn Asn Asp Asn Trp Val Lys Gln Asp Glu Val Ile Thr Leu
      210          215          220
Leu Ser Asn Ile Arg Ser Asn Arg Cys Lys Ile Tyr Ser Leu Leu Gly
      225          230          235          240
Ser Ser His Asp Leu Ser Glu Asn Leu Val Val Leu Arg Asn Phe Tyr
      245          250          255
Gln Ser Val Thr Lys Ala Ala Ile Ala Met Asp Asn Asp His Leu Asp
      260          265          270
Ile Asp Val Asp Ile Thr Glu Pro Ser Phe Glu His Leu Thr Ile Ala
      275          280          285

```

004240 23552350

Thr Val Asn Glu Arg Arg Met Arg Ile Glu Ile Glu Asn Gln Ala Ile
 290 295 300

Ser Leu Ser
 305

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 360 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Lys Phe Gly Asn Phe Leu Leu Thr Tyr Gln Pro Pro Gln Phe Ser
 1 5 10 15
 Gln Thr Glu Val Met Lys Arg Leu Val Lys Leu Gly Arg Ile Ser Glu
 20 25 30
 Glu Cys Gly Phe Asp Thr Val Trp Leu Leu Glu His His Phe Thr Glu
 35 40 45
 Phe Gly Leu Leu Gly Asn Pro Tyr Val Ala Ala Ala Tyr Leu Leu Gly
 50 55 60
 Ala Thr Lys Lys Leu Asn Val Gly Thr Ala Ala Ile Val Leu Pro Thr
 65 70 75 80
 Ala His Pro Val Arg Gln Leu Glu Asp Val Asn Leu Leu Asp Gln Met
 85 90 95
 Ser Lys Gly Arg Phe Arg Phe Gly Ile Cys Arg Gly Leu Tyr Asn Lys
 100 105 110
 Asp Phe Arg Val Phe Gly Thr Asp Met Asn Asn Ser Arg Ala Leu Ala
 115 120 125
 Glu Cys Trp Tyr Gly Leu Ile Lys Asn Gly Met Thr Glu Gly Tyr Met
 130 135 140
 Glu Ala Asp Asn Glu His Ile Lys Phe His Lys Val Lys Val Asn Pro
 145 150 155 160
 Ala Ala Tyr Ser Arg Gly Gly Ala Pro Val Tyr Val Val Ala Glu Ser
 165 170 175
 Ala Ser Thr Thr Glu Trp Ala Ala Gln Phe Gly Leu Pro Met Ile Leu
 180 185 190
 Ser Trp Ile Ile Asn Thr Asn Glu Lys Lys Ala Gln Leu Glu Leu Tyr
 195 200 205
 Asn Glu Val Ala Gln Glu Tyr Gly His Asp Ile His Asn Ile Asp His
 210 215 220
 Cys Leu Ser Tyr Ile Thr Ser Val Asp His Asp Ser Ile Lys Ala Lys
 225 230 235 240
 Glu Ile Cys Arg Lys Phe Leu Gly His Trp Tyr Asp Ser Tyr Val Asn
 245 250 255
 Ala Thr Thr Ile Phe Asp Asp Ser Asp Gln Thr Arg Gly Tyr Asp Phe
 260 265 270

004240 1955555550

Asn Lys Gly Gln Trp Arg Asp Phe Val Leu Lys Gly His Lys Asp Thr
 275 280 285
 Asn Arg Arg Ile Asp Tyr Ser Tyr Glu Ile Asn Pro Val Gly Thr Pro
 290 295 300
 Gln Glu Cys Ile Asp Ile Ile Gln Lys Asp Ile Asp Ala Thr Gly Ile
 305 310 315 320
 Ser Asn Ile Cys Cys Gly Phe Glu Ala Asn Gly Thr Val Asp Glu Ile
 325 330 335
 Ile Ala Ser Met Lys Leu Phe Gln Ser Asp Val Met Pro Phe Leu Lys
 340 345 350
 Glu Lys Gln Arg Ser Leu Leu Tyr
 355 360

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 327 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Lys Phe Gly Leu Phe Phe Leu Asn Phe Ile Asn Ser Thr Thr Val
 1 5 10 15
 Gln Glu Gln Ser Ile Val Arg Met Gln Glu Ile Thr Glu Tyr Val Asp
 20 25 30
 Lys Leu Asn Phe Glu Gln Ile Leu Val Tyr Glu Asn His Phe Ser Asp
 35 40 45
 Asn Gly Val Val Gly Ala Pro Leu Thr Val Ser Gly Phe Leu Leu Gly
 50 55 60
 Leu Thr Glu Lys Ile Lys Ile Gly Ser Leu Asn His Ile Ile Thr Thr
 65 70 75 80
 His His Pro Val Ala Ile Ala Glu Glu Ala Cys Leu Leu Asp Gln Leu
 85 90 95
 Ser Glu Gly Arg Phe Ile Leu Gly Phe Ser Asp Cys Glu Lys Lys Asp
 100 105 110
 Glu Met His Phe Phe Asn Arg Pro Val Glu Tyr Gln Gln Gln Leu Phe
 115 120 125
 Glu Glu Cys Tyr Glu Ile Ile Asn Asp Ala Leu Thr Thr Gly Tyr Cys
 130 135 140
 Asn Pro Asp Asn Asp Phe Tyr Ser Phe Pro Lys Ile Ser Val Asn Pro
 145 150 155 160
 His Ala Tyr Thr Pro Gly Gly Pro Arg Lys Tyr Val Thr Ala Thr Ser
 165 170 175
 His His Ile Val Glu Trp Ala Ala Lys Lys Gly Ile Pro Leu Ile Phe
 180 185 190
 Lys Trp Asp Asp Ser Asn Asp Val Arg Tyr Glu Tyr Ala Glu Arg Tyr
 195 200 205

Lys Ala Val Ala Asp Lys Tyr Asp Val Asp Leu Ser Glu Ile Asp His
 210 215 220
 Gln Leu Met Ile Leu Val Asn Tyr Asn Glu Asp Ser Asn Lys Ala Lys
 225 230 235 240
 Gln Glu Thr Arg Ala Phe Ile Ser Asp Tyr Val Leu Glu Met His Pro
 245 250 255
 Asn Glu Asn Phe Glu Asn Lys Leu Glu Glu Ile Ile Ala Glu Asn Ala
 260 265 270
 Val Gly Asn Tyr Thr Glu Cys Ile Thr Ala Ala Lys Leu Ala Ile Glu
 275 280 285
 Lys Cys Gly Ala Lys Ser Val Leu Leu Ser Phe Glu Pro Met Asn Asp
 290 295 300
 Leu Met Ser Gln Lys Asn Val Ile Asn Ile Val Asp Asp Asn Ile Lys
 305 310 315 320
 Lys Tyr His Met Glu Tyr Thr
 325

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 394 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Lys Gly Ile Lys Glu Tyr Asp Ser Ser Ala Ala Ile Leu Ser Asn
 1 5 10 15
 Ile Ile Leu Arg Ser Lys Thr Gly Met Thr Ser Tyr Val Asp Lys Gln
 20 25 30
 Glu Ile Thr Ala Ser Ser Glu Ile Asp Asp Leu Ile Phe Ser Ser Asp
 35 40 45
 Pro Leu Val Trp Ser Tyr Asp Glu Gln Glu Lys Ile Arg Lys Lys Leu
 50 55 60
 Val Leu Asp Ala Phe Arg Asn His Tyr Lys His Cys Arg Glu Tyr Arg
 65 70 75 80
 His Tyr Cys Gln Ala His Lys Val Asp Asp Asn Ile Thr Glu Ile Asp
 85 90 95
 Asp Ile Pro Val Phe Pro Thr Ser Val Phe Lys Phe Thr Arg Leu Leu
 100 105 110
 Thr Ser Gln Glu Asn Glu Ile Glu Ser Trp Phe Thr Ser Ser Gly Thr
 115 120 125
 Asn Gly Leu Lys Ser Gln Val Ala Arg Asp Arg Leu Ser Ile Glu Arg
 130 135 140
 Leu Leu Gly Ser Val Ser Tyr Gly Met Lys Tyr Val Gly Ser Trp Phe
 145 150 155 160
 Asp His Gln Ile Glu Leu Val Asn Leu Gly Pro Asp Arg Phe Asn Ala
 165 170 175

His Asn Ile Trp Phe Lys Tyr Val Met Ser Leu Val Glu Leu Leu Tyr
 180 185 190
 Pro Thr Thr Phe Thr Val Thr Glu Glu Arg Ile Asp Phe Val Lys Thr
 195 200 205
 Leu Asn Ser Leu Glu Arg Ile Lys Asn Gln Gly Lys Asp Leu Cys Leu
 210 215 220
 Ile Gly Ser Pro Tyr Phe Ile Tyr Leu Leu Cys His Tyr Met Lys Asp
 225 230 235 240
 Lys Lys Ile Ser Phe Ser Gly Asp Lys Ser Leu Tyr Ile Ile Thr Gly
 245 250 255
 Gly Gly Trp Lys Ser Tyr Glu Lys Glu Ser Leu Lys Arg Asp Asp Phe
 260 265 270
 Asn His Leu Leu Phe Asp Thr Phe Asn Leu Ser Asp Ile Ser Gln Ile
 275 280 285
 Arg Asp Ile Phe Asn Gln Val Glu Leu Asn Thr Cys Phe Phe Glu Asp
 290 295 300
 Glu Met Gln Arg Lys His Val Pro Pro Trp Val Tyr Ala Arg Ala Leu
 305 310 315 320
 Asp Pro Glu Thr Leu Lys Pro Val Pro Asp Gly Thr Pro Gly Leu Met
 325 330 335
 Ser Tyr Met Asp Ala Ser Ala Thr Ser Tyr Pro Ala Phe Ile Val Thr
 340 345 350
 Asp Asp Val Gly Ile Ile Ser Arg Glu Tyr Gly Lys Tyr Pro Gly Val
 355 360 365
 Leu Val Glu Ile Leu Arg Arg Val Asn Thr Arg Thr Gln Lys Gly Cys
 370 375 380
 Ala Leu Ser Leu Thr Glu Ala Phe Asp Ser
 385 390

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3098 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: pASK75

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: vector

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 542..672
- (D) OTHER INFORMATION: /function= "beta-la promoter"
 /label= beta-la
 /citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:673..1530
- (D) OTHER INFORMATION:/product= "beta-la"
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1543..2163
- (D) OTHER INFORMATION:/product= "tetR"
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:2713..2950
- (D) OTHER INFORMATION:/function= "ORI"
/label= ORI
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:2976..3073
- (D) OTHER INFORMATION:/function= "p tetA promoter"
/citation= ([1])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Skerra, A
- (B) TITLE: Use of the tetracycline promoter for the
tightly regulated production of a murine antibody
fragment in Escherichia coli
- (C) JOURNAL: Gene
- (D) VOLUME: 151
- (E) ISSUE: 1-2
- (F) PAGES: 131-135
- (G) DATE: 30-DEC-1994
- (K) RELEVANT RESIDUES IN SEQ ID NO: 9: FROM 1 TO 3098

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```

AGCTTGACCT GTGAAGTGAA AAATGGCGCA CATTGTGCGA CATTTTTTTT GTCTGCCGTT      60
TACCGCTACT GCGTCACGGA TCTCCACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT      120
GTGGTGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC      180
GCTTTCTTCC CTTCTTTTCT CGCCACGTTT GCCGGCTTTC CCCGTCAAGC TCTAAATCGG      240
GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT      300
TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG      360
TTGGAGTCCA CGTCTTTTAA TAGTGGA CTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT      420
ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA      480
AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT      540
TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA      600
CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA      660
AAAAGGAAGA GT ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC      708
          Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro
          395                400                405

```

TTT TTT GCG GCA TTT TGC CTT CCT GTT TTT GCT CAC CCA GAA ACG CTG	756
Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu	
410 415 420	
GTG AAA GTA AAA GAT GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC	804
Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr	
425 430 435	
ATC GAA CTG GAT CTC AAC AGC GGT AAG ATC CTT GAG AGT TTT CGC CCC	852
Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro	
440 445 450	
GAA GAA CGT TTT CCA ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC	900
Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly	
455 460 465 470	
GCG GTA TTA TCC CGT ATT GAC GCC GGG CAA GAG CAA CTC GGT CGC CGC	948
Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg	
475 480 485	
ATA CAC TAT TCT CAG AAT GAC TTG GTT GAG TAC TCA CCA GTC ACA GAA	996
Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu	
490 495 500	
AAG CAT CTT ACG GAT GGC ATG ACA GTA AGA GAA TTA TGC AGT GCT GCC	1044
Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala	
505 510 515	
ATA ACC ATG AGT GAT AAC ACT GCG GCC AAC TTA CTT CTG ACA ACG ATC	1092
Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Thr Thr Ile	
520 525 530	
GGA GGA CCG AAG GAG CTA ACC GCT TTT TTG CAC AAC ATG GGG GAT CAT	1140
Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His	
535 540 545 550	
GTA ACT CGC CTT GAT CGT TGG GAA CCG GAG CTG AAT GAA GCC ATA CCA	1188
Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro	
555 560 565	
AAC GAC GAG CGT GAC ACC ACG ATG CCT GTA GCA ATG GCA ACA ACG TTG	1236
Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu	
570 575 580	
CGC AAA CTA TTA ACT GGC GAA CTA CTT ACT CTA GCT TCC CGG CAA CAA	1284
Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln	
585 590 595	
TTG ATA GAC TGG ATG GAG GCG GAT AAA GTT GCA GGA CCA CTT CTG CGC	1332
Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg	
600 605 610	
TCG GCC CTT CCG GCT GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC GGT	1380
Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly	
615 620 625 630	
GAG CGT GGC TCT CGC GGT ATC ATT GCA GCA CTG GGG CCA GAT GGT AAG	1428
Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys	
635 640 645	
CCC TCC CGT ATC GTA GTT ATC TAC ACG ACG GGG AGT CAG GCA ACT ATG	1476
Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met	
650 655 660	
GAT GAA CGA AAT AGA CAG ATC GCT GAG ATA GGT GCC TCA CTG ATT AAG	1524
Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys	
665 670 675	

CAT TGG TAGGAATTAA TG ATG TCT CGT TTA GAT AAA AGT AAA GTG ATT His Trp Met Ser Arg Leu Asp Lys Ser Lys Val Ile 680 1 5 10	1572
AAC AGC GCA TTA GAG CTG CTT AAT GAG GTC GGA ATC GAA GGT TTA ACA Asn Ser Ala Leu Glu Leu Leu Asn Glu Val Gly Ile Glu Gly Leu Thr 15 20 25	1620
ACC CGT AAA CTC GCC CAG AAG CTA GGT GTA GAG CAG CCT ACA TTG TAT Thr Arg Lys Leu Ala Gln Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr 30 35 40	1668
TGG CAT GTA AAA AAT AAG CGG GCT TTG CTC GAC GCC TTA GCC ATT GAG Trp His Val Lys Asn Lys Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu 45 50 55	1716
ATG TTA GAT AGG CAC CAT ACT CAC TTT TGC CCT TTA GAA GGG GAA AGC Met Leu Asp Arg His His Thr His Phe Cys Pro Leu Glu Gly Glu Ser 60 65 70	1764
TGG CAA GAT TTT TTA CGT AAT AAC GCT AAA AGT TTT AGA TGT GCT TTA Trp Gln Asp Phe Leu Arg Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu 75 80 85 90	1812
CTA AGT CAT CGC GAT GGA GCA AAA GTA CAT TTA GGT ACA CGG CCT ACA Leu Ser His Arg Asp Gly Ala Lys Val His Leu Gly Thr Arg Pro Thr 95 100 105	1860
GAA AAA CAG TAT GAA ACT CTC GAA AAT CAA TTA GCC TTT TTA TGC CAA Glu Lys Gln Tyr Glu Thr Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln 110 115 120	1908
CAA GGT TTT TCA CTA GAG AAT GCA TTA TAT GCA CTC AGC GCA GTG GGG Gln Gly Phe Ser Leu Glu Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly 125 130 135	1956
CAT TTT ACT TTA GGT TGC GTA TTG GAA GAT CAA GAG CAT CAA GTC GCT His Phe Thr Leu Gly Cys Val Leu Glu Asp Gln Glu His Gln Val Ala 140 145 150	2004
AAA GAA GAA AGG GAA ACA CCT ACT ACT GAT AGT ATG CCG CCA TTA TTA Lys Glu Glu Arg Glu Thr Pro Thr Thr Asp Ser Met Pro Pro Leu Leu 155 160 165 170	2052
CGA CAA GCT ATC GAA TTA TTT GAT CAC CAA GGT GCA GAG CCA GCC TTC Arg Gln Ala Ile Glu Leu Phe Asp His Gln Gly Ala Glu Pro Ala Phe 175 180 185	2100
TTA TTC GGC CTT GAA TTG ATC ATA TGC GGA TTA GAA AAA CAA CTT AAA Leu Phe Gly Leu Glu Leu Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys 190 195 200	2148
TGT GAA AGT GGG TCT TAAAAGCAGC ATAACCTTTT TCCGTGATGG TAACTTCACT Cys Glu Ser Gly Ser 205	2203
AGTTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC	2263
GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG	2323
ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG	2383
TGGTTTGTTC GCCGGATCAA GAGCTACCAA CTCTTTTTC GAAGGTAACCT GGCTTCAGCA	2443
GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA	2503
ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA	2563

GTGGCGATAA GTCGTGTCCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC 2623
 AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA 2683
 CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA 2743
 AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC 2803
 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC TGACTTGAGC 2863
 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG 2923
 CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTTGCTCA CATGACCCGA CACCATCGAA 2983
 TGGCCAGATG ATTAATTCCT AATTTTTGTT GACACTCTAT CATTGATAGA GTTATTTTAC 3043
 CACTCCCTAT CAGTGATAGA GAAAAGTGAA ATGAATAGTT CGACAAAAAT CTAGA 3098

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 286 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
 1 5 10 15
 Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
 20 25 30
 Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
 35 40 45
 Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
 50 55 60
 Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
 65 70 75 80
 Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
 85 90 95
 Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
 100 105 110
 Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
 115 120 125
 Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
 130 135 140
 Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
 145 150 155 160
 Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
 165 170 175
 Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
 180 185 190
 Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
 195 200 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
 210 215 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
 225 230 235 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
 245 250 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
 260 265 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
 275 280 285

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 207 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Ser Arg Leu Asp Lys Ser Lys Val Ile Asn Ser Ala Leu Glu Leu
 1 5 10 15

Leu Asn Glu Val Gly Ile Glu Gly Leu Thr Thr Arg Lys Leu Ala Gln
 20 25 30

Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr Trp His Val Lys Asn Lys
 35 40 45

Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu Met Leu Asp Arg His His
 50 55 60

Thr His Phe Cys Pro Leu Glu Gly Glu Ser Trp Gln Asp Phe Leu Arg
 65 70 75 80

Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu Leu Ser His Arg Asp Gly
 85 90 95

Ala Lys Val His Leu Gly Thr Arg Pro Thr Glu Lys Gln Tyr Glu Thr
 100 105 110

Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln Gln Gly Phe Ser Leu Glu
 115 120 125

Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly His Phe Thr Leu Gly Cys
 130 135 140

Val Leu Glu Asp Gln Glu His Gln Val Ala Lys Glu Glu Arg Glu Thr
 145 150 155 160

Pro Thr Thr Asp Ser Met Pro Pro Leu Leu Arg Gln Ala Ile Glu Leu
 165 170 175

Phe Asp His Gln Gly Ala Glu Pro Ala Phe Leu Phe Gly Leu Glu Leu
 180 185 190

Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys Cys Glu Ser Gly Ser
 195 200 205

CLAIMS

1. A method for the determination of a tetracycline in a sample characterized in that
 - the sample is brought into contact with prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,
 - detecting the luminescence emitted from the intact cells, and
 - comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline
 - wherein a detectable luminescence higher than a luminescence of the control indicates the presence of tetracycline in the sample.
2. The method according to claim 1 characterized in that the cells are *Escherichia coli*.
3. The method according to claim 1 or 2 characterized in that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*.
4. The method according to claim 3 characterized in that the DNA vector is the plasmid pTetLux1 (SEQ ID NO: 3).
5. The method according to claim 1 or 2 characterized in that
 - the DNA vector is a plasmid containing the insect luciferase gene (SEQ ID NO: 1), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn10*, and that

01-12-1999

50

- D-luciferin is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells.
6. The method according to claim 5 characterized in that the DNA vector is the plasmid pTetLuc1 (SEQ ID NO: 1).
7. The method according to any of the claims 1 - 6 characterized in that the sensitivity of the analysis with respect to the tetracycline is controlled by
- increasing or decreasing the concentration of divalent metal ions, e.g. magnesium ions, or
 - adjusting the pH, or
 - combined adjusting of the divalent metal ion concentration and the pH.
8. The method according to any of the claims 1 - 6 characterized in that the sensitivity of the analysis with respect to the tetracycline derivative is increased by the use of cells which are especially antibiotic sensitive mutant strains.
9. The method according to any of the claims 1 - 8 characterized in that the luminescence is measured using an X-ray or polaroid film, a CCD-camera, a liquid scintillation counter or a luminometer.
10. The method according to any of the claims 1 - 9 characterized in that the sample to be analyzed is milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma, whole blood or the like.
11. A recombinant prokaryotic cell characterized in that it encompasses a DNA vector including a nucleotide sequence encoding a light producing enzyme,

01-12-1999

51

tetracycline repressor and tetracycline promoter, and that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promoter (TetA) (SEQ ID NO: 9) from *Tn10*.

12. The cell according to claim 11 characterized in that it is *Escherichia coli*.

13. The cell according to claim 11 or 12, characterized in that it is in dried form, e.g. in lyophilized form.

14. A plasmid characterized in that it comprises the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promoter (TetA) (SEQ ID NO: 9) from *Tn10*.

15. A plasmid according to claim 14 characterized in that it is pTetLux1 (SEQ ID NO: 3).

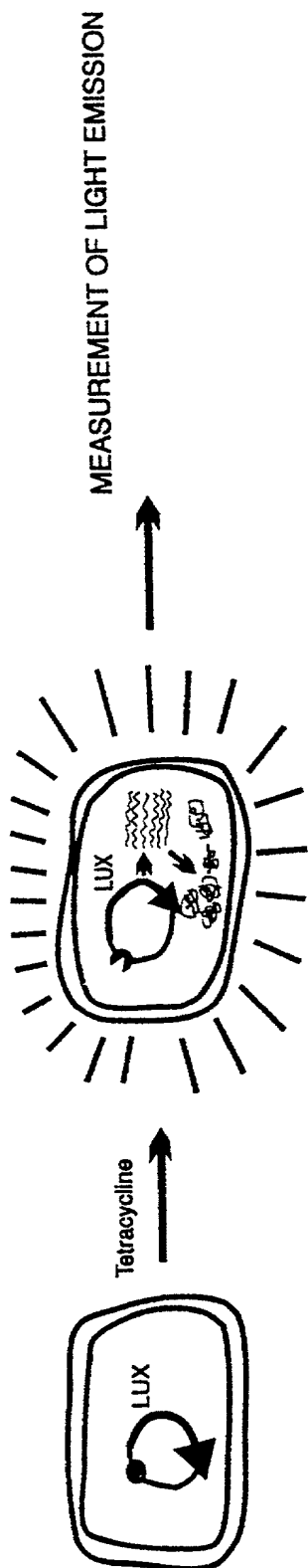


FIG. 1a

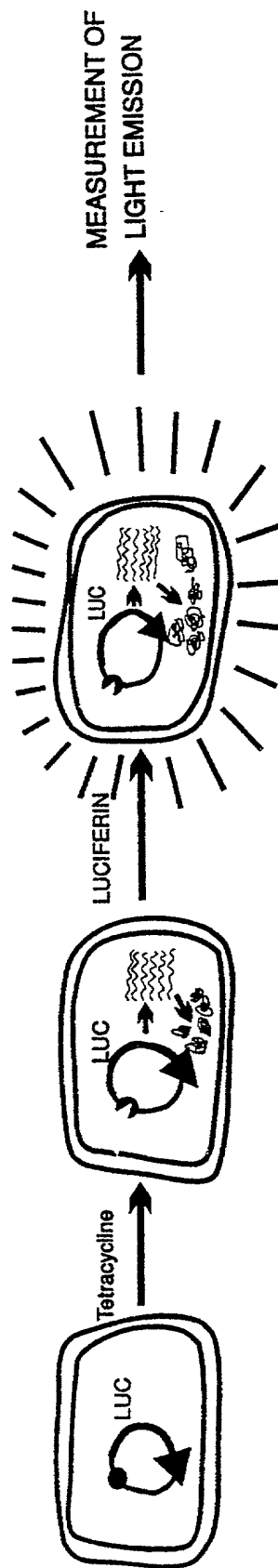


FIG. 1b

2/11

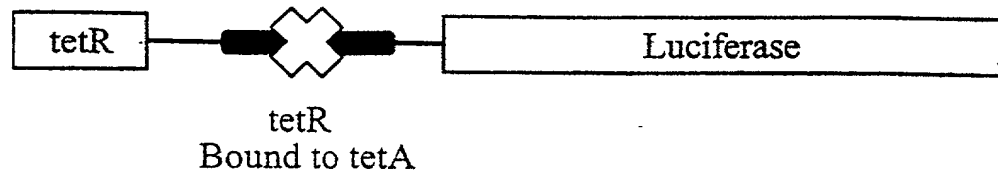
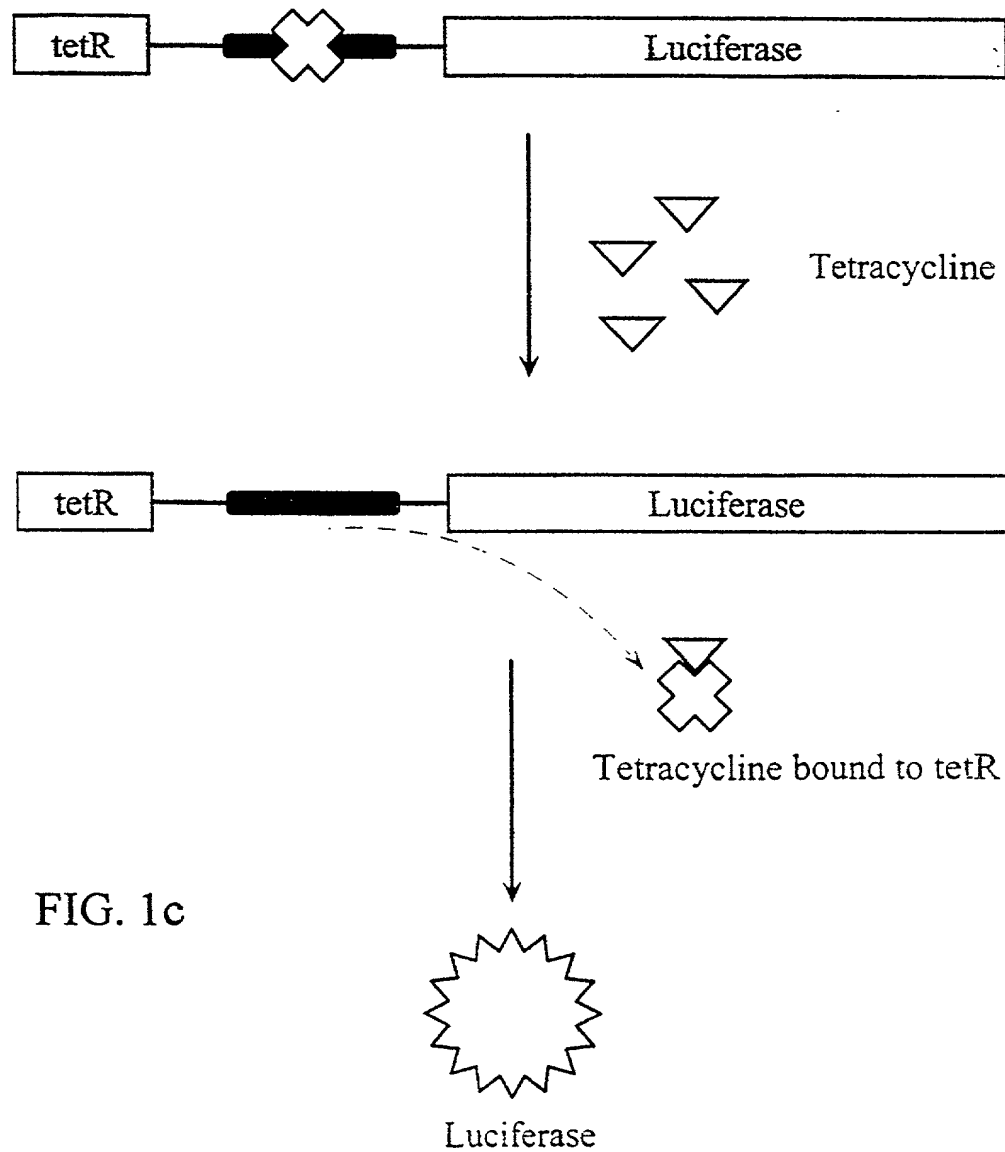
A. No Protein ExpressionB. Protein Expression

FIG. 1c

3/11

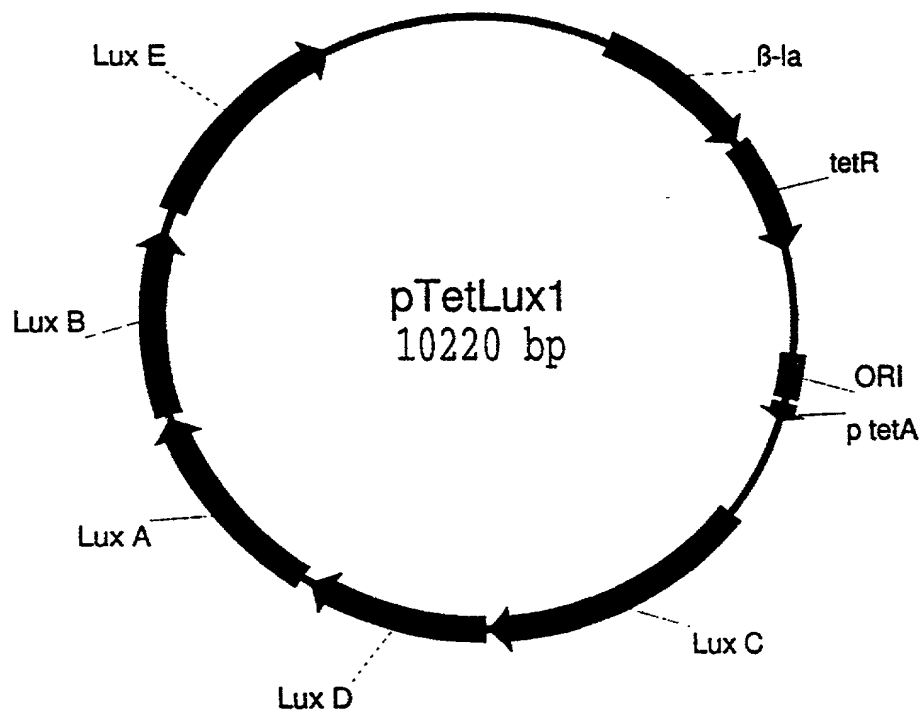


FIG. 2

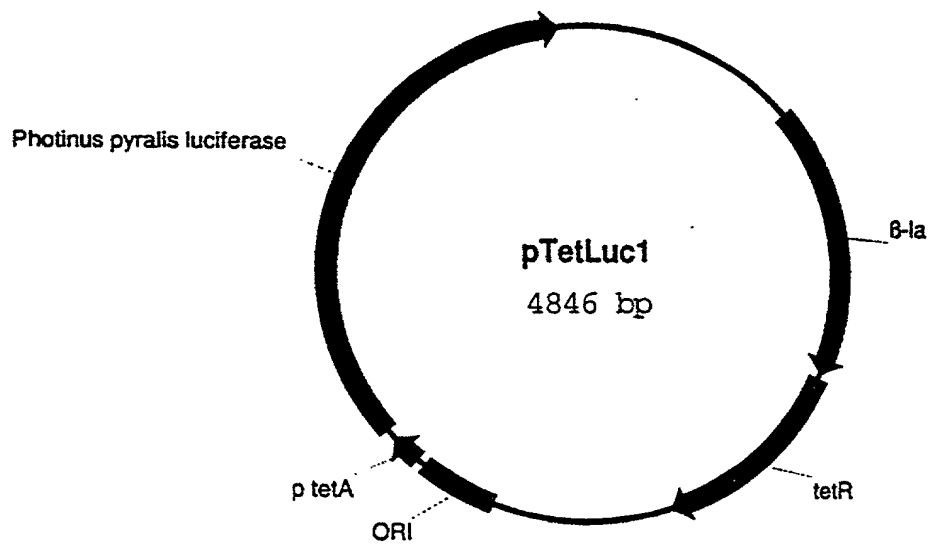


FIG. 3

FIG. 4a

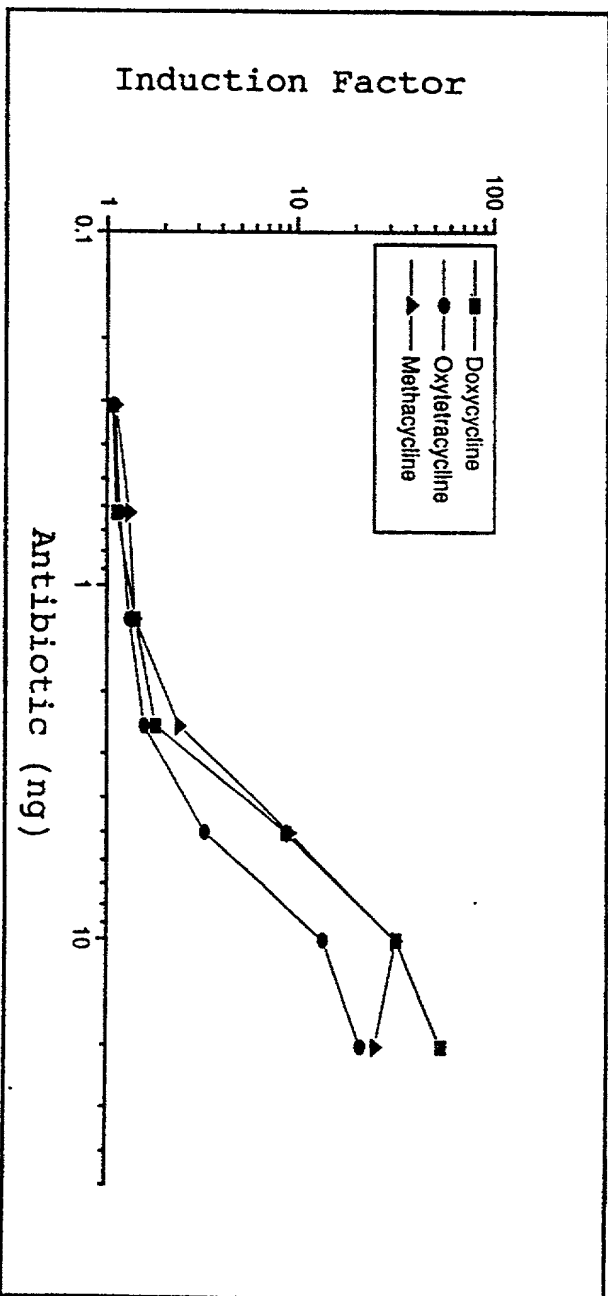
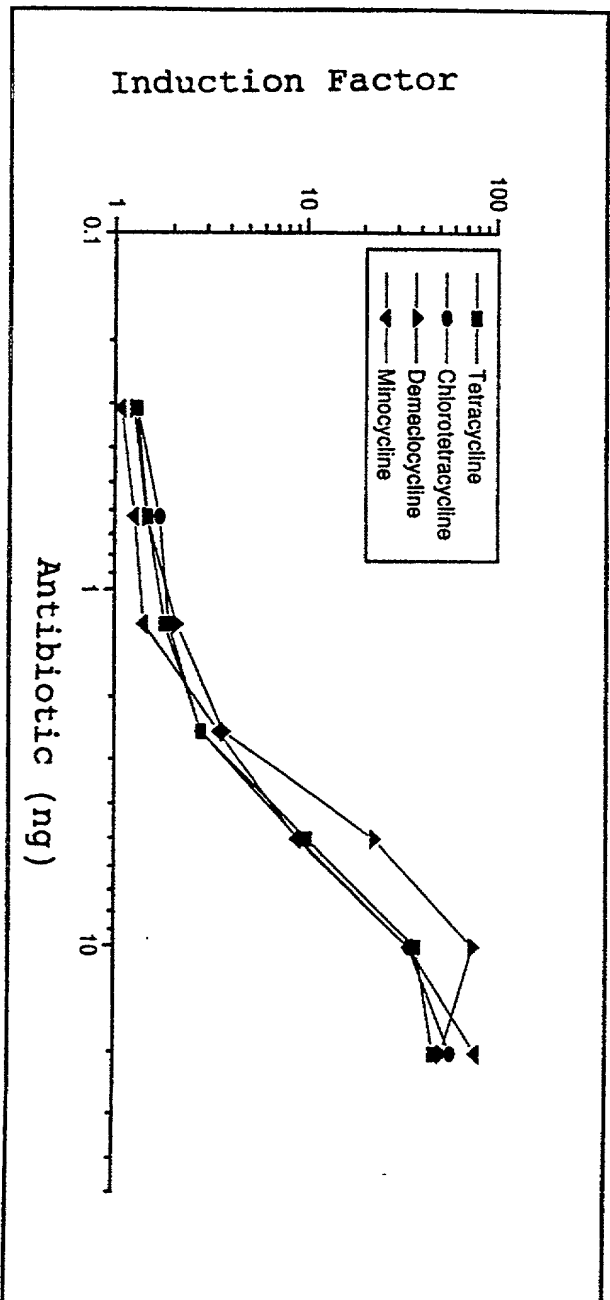


FIG. 4b



09/529967 042400

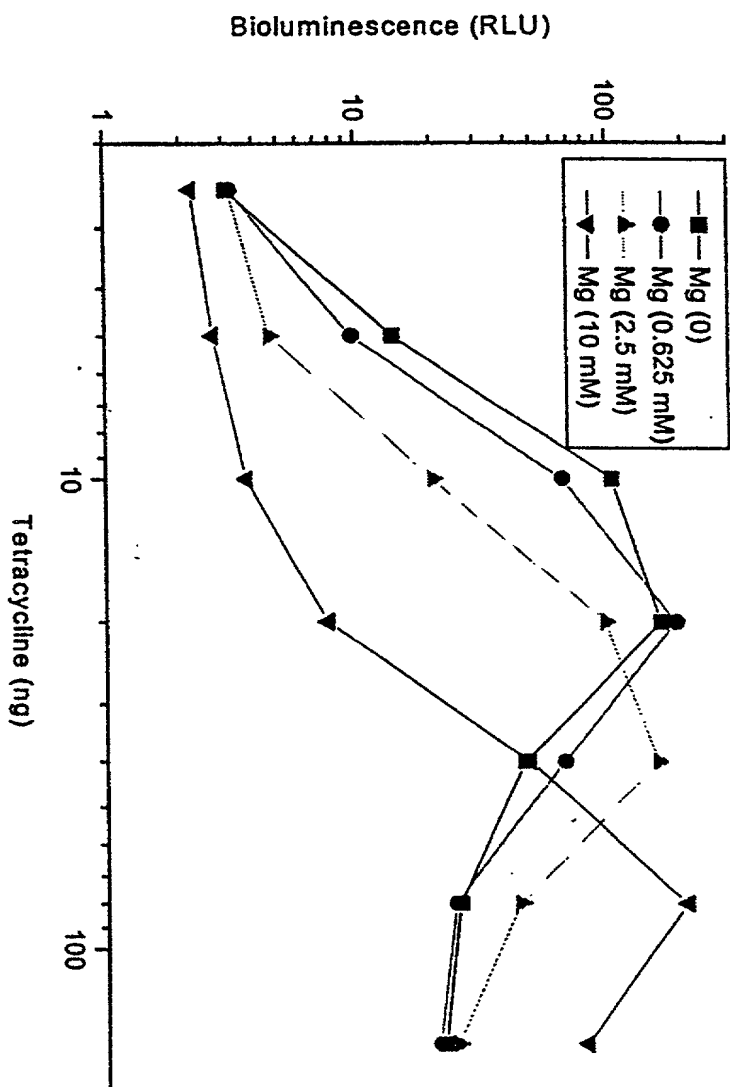


FIG. 5

09/529967 042400

09/529967

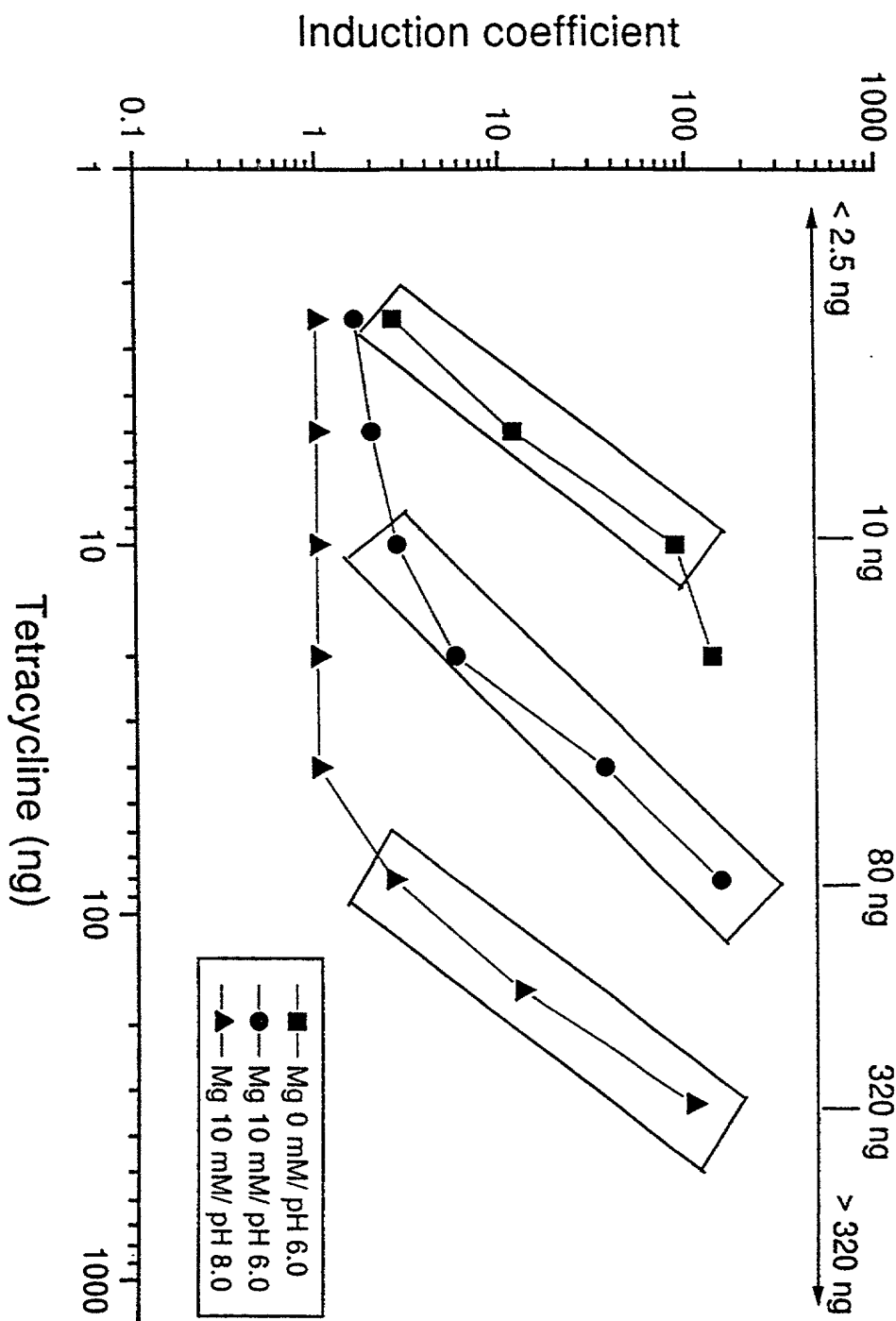


FIG. 6

09/529967 042400

Induction Factor

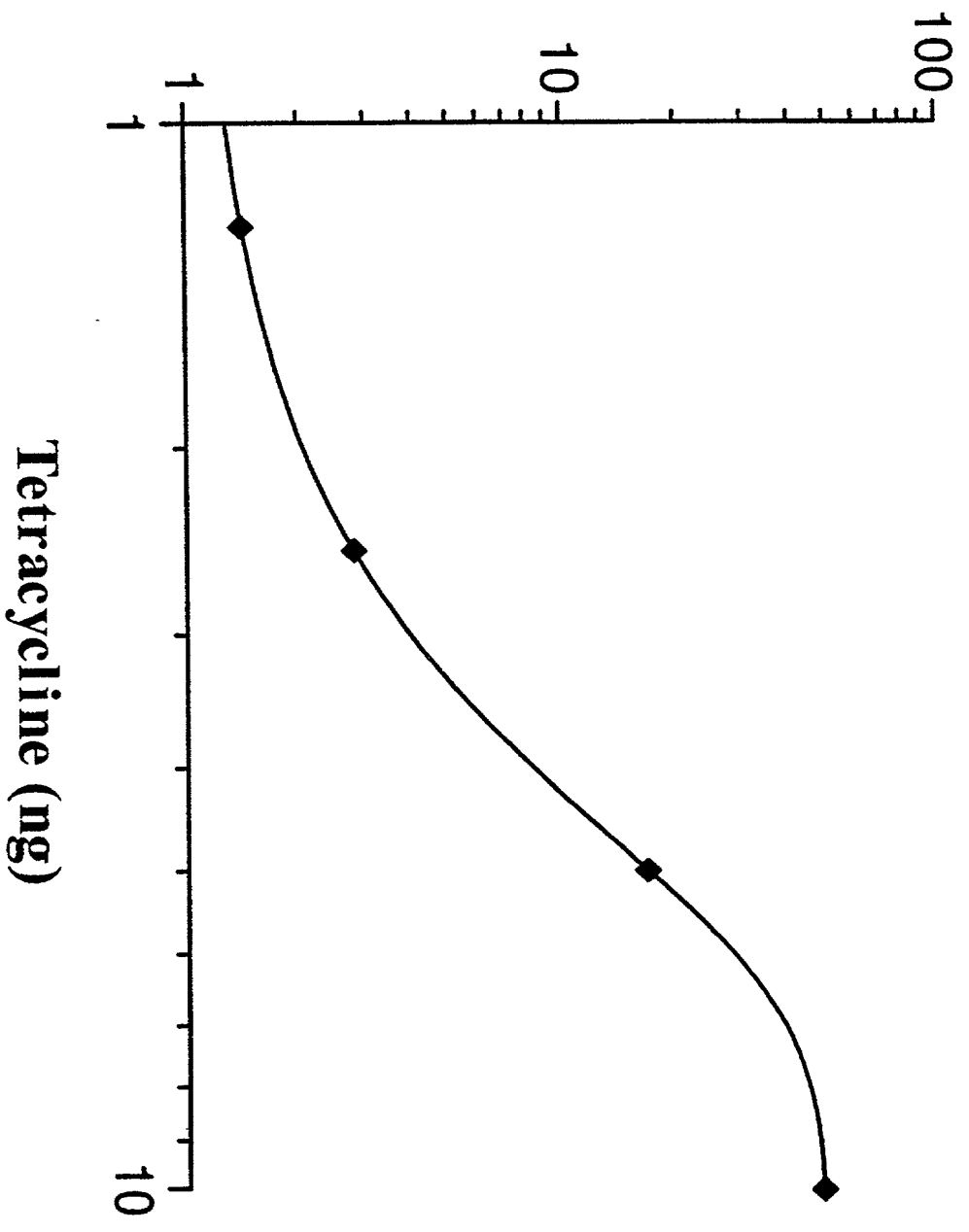


FIG. 7

7/11

03623367 042400

8/11

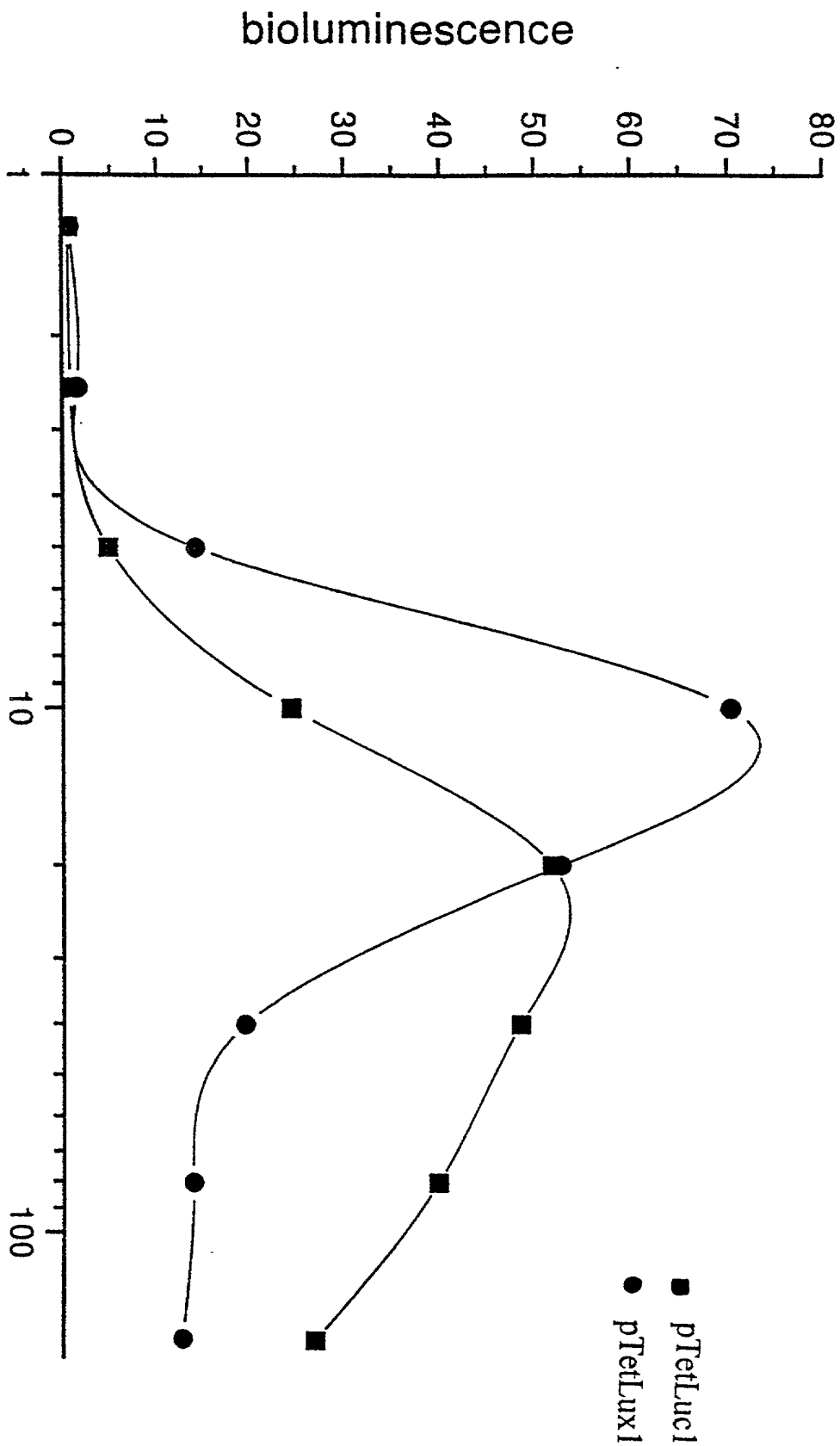


FIG. 8

tetracycline ng/well

■ pTetLuc1
● pTetLux1

09/529967 042400

09/529967

Induction Factor

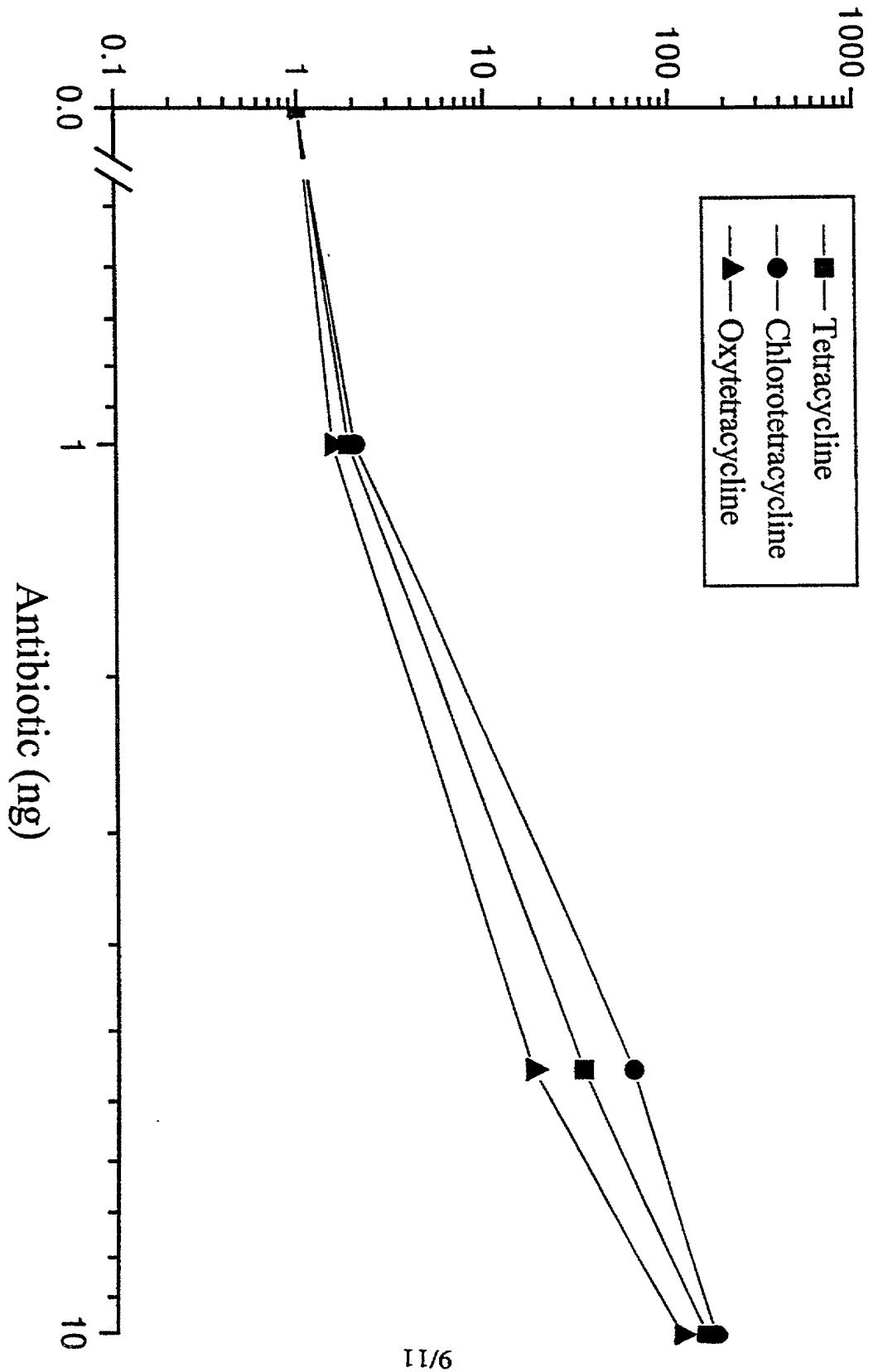
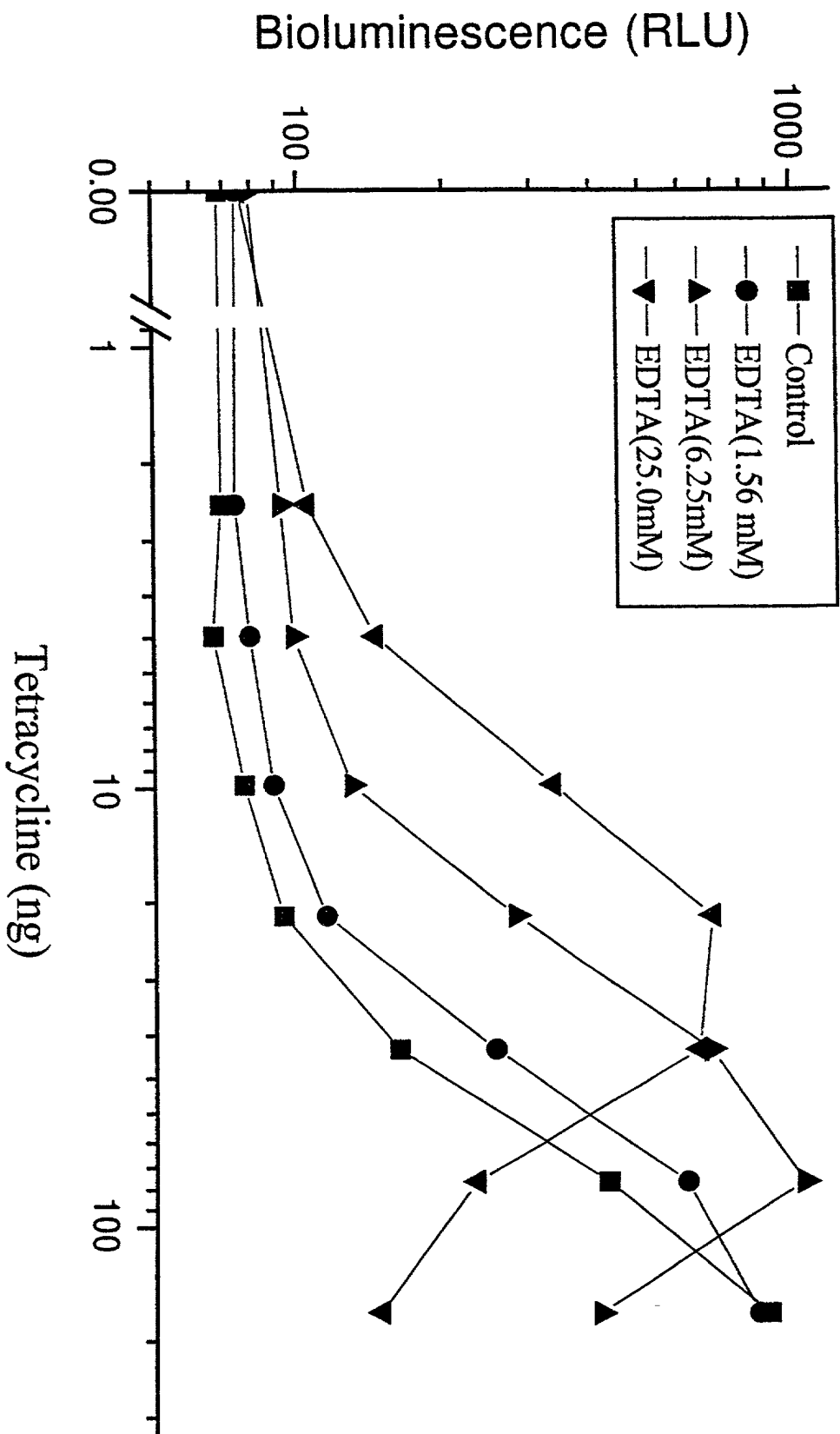


FIG. 9

FIG.10



11/11

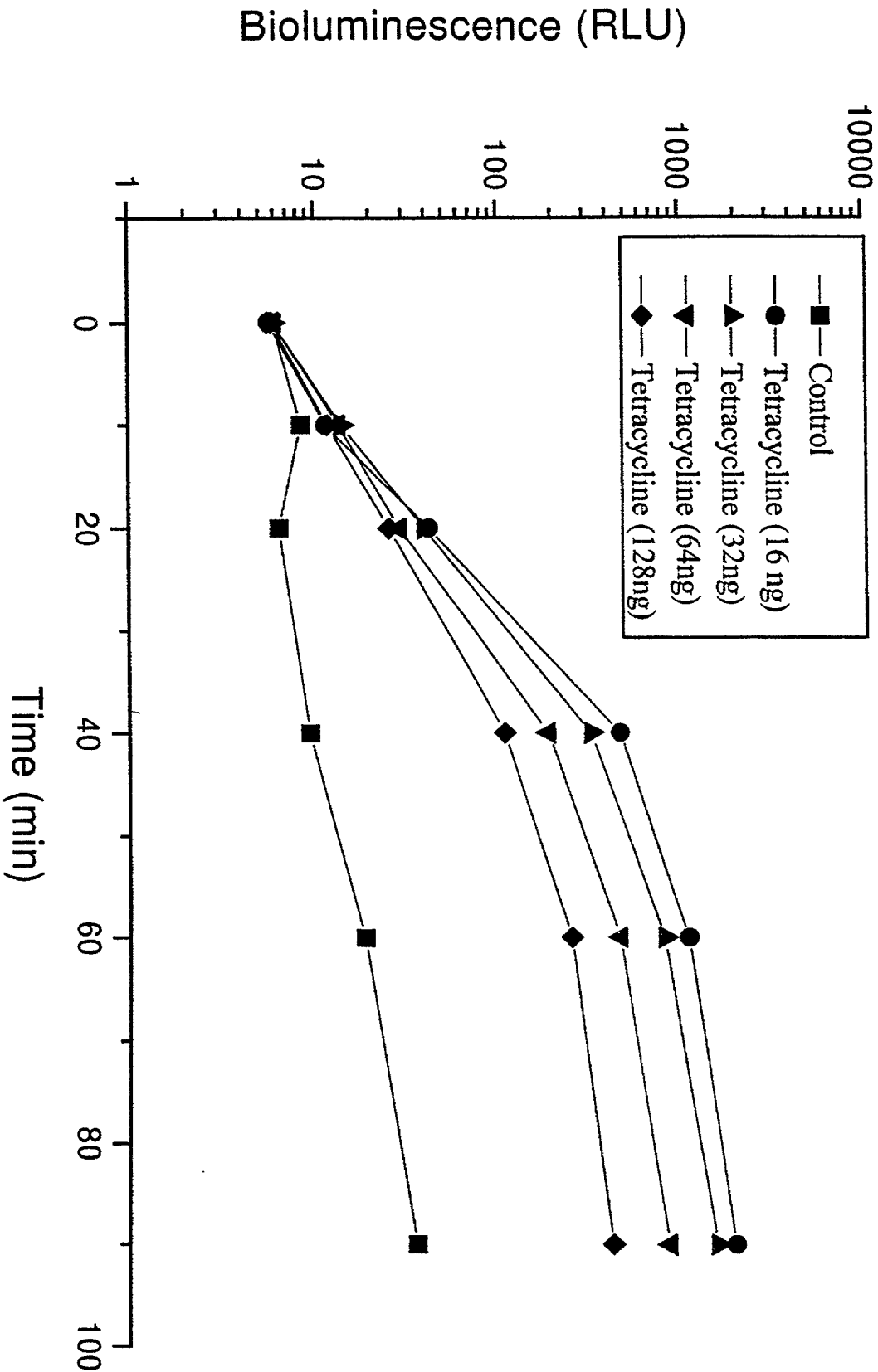


FIG.11

Attorney Docket No. _____

**Declaration and Power of Attorney
For Patent Application
(Sole/Joint)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought, on the invention entitled "Tetracycline assay method"

_____ the specification of which (Check One)

_____ is attached hereto.

X was filed on November 11, 1998 as

[] Application Serial No. _____

[X] International Application No. PCT/FI98/00873

and was amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

			Priority Claimed	
<u>974235</u>	<u>Finland</u>	<u>14/11/1997</u>	Yes: <u>X</u>	No: _____
(Number)	(Country)	(Day/Month/Year Filed)		
_____	_____	_____	Yes: _____	No: _____
(Number)	(Country)	(Day/Month/Year Filed)		
_____	_____	_____	Yes: _____	No: _____
(Number)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose

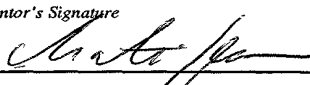
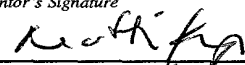
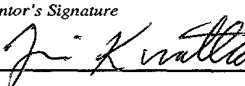
material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status)

I or we hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to ROTHWELL, FIGG, ERNST & KURZ, P.C., 555 13th Street, N.W., Washington, D.C. 20004

G. Franklin Rothwell, Reg. No. 18,125	Michael G. Sullivan, Reg. No. 35,377
E. Anthony Figg, Reg. No. 27,195	Mark I. Bowditch, Reg. No. 40,315
Barbara G. Ernst, Reg. No. 30,377	Robert J. Jondle, Reg. No. 33,915
George R. Repper, Reg. No. 31,414	Moon Soo Lee, Reg. No. 37,377
Bart G. Newland, Reg. No. 31,282	Kenneth M. Fagin, Reg. No. 37,615
Vincent M. DeLuca, Reg. No. 32,408	Stephen B. Parker, Reg. No. 36,631
Celine Jimenez Crowson, Reg. No. 40,357	Michael J. Donnelly, Reg. No. 38,126
Joseph A. Hynds, Reg. No. 34,627	Minaksi Bhatt, Reg. No. 35,447

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole or First Inventor	Inventor's Signature	Date
Matti KORPELA		18.4.2000
Residence	Citizenship	
Maijamäentie 13, FIN-21100 Naantali, Finland	FIX	Finnish
Post Office Address	same as residence	
Full Name of Second Joint Inventor, if any	Inventor's Signature	Date
Matti KARP		17.4.2000
Residence	Citizenship	
Kampakatu 1, FIN-20660 Littoinen, Finland	FIX	Finnish
Post Office Address	same as residence	
Full Name of Third Joint Inventor, if any	Inventor's Signature	Date
Jussi KURITTU		17.4.2000
Residence	Citizenship	
Tuureporinkatu 13 D073, FIN-20100 Turku, Finland	FIX	Finnish
Post Office Address	same as residence	
Full Name of Fourth Joint Inventor, if any	Inventor's Signature	Date
Residence	Citizenship	
Post Office Address		